



US 20040115634A1

(19) **United States**

(12) **Patent Application Publication**

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(10) **Pub. No.: US 2004/0115634 A1**

(43) **Pub. Date: Jun. 17, 2004**

(54) MODULATION OF STAT 6 EXPRESSION

(22) Filed: Dec. 11, 2002

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(21) Appl. No.: 10/317,391

Publication Classification

(51) Int. Cl.<sup>7</sup> ..... C12Q 1/68; C07H 21/04;  
A61K 48/00  
(52) U.S. Cl. .... 435/6; 514/44; 536/23.2

(57) ABSTRACT

Compounds, compositions and methods are provided for modulating the expression of STAT 6. The compositions comprise oligonucleotides, targeted to nucleic acid encoding STAT 6. Methods of using these compounds for modulation of STAT 6 expression and for diagnosis and treatment of disease associated with expression of STAT 6 are provided.

## MODULATION OF STAT 6 EXPRESSION

### FIELD OF THE INVENTION

[0001] The present invention provides compositions and methods for modulating the expression of STAT 6. In particular, this invention relates to compounds, particularly oligonucleotide compounds, which, in preferred embodiments, hybridize with nucleic acid molecules encoding STAT 6. Such compounds are shown herein to modulate the expression of STAT 6.

### BACKGROUND OF THE INVENTION

[0002] Cytokines function as protein mediators that play a critical role in host defense and serve as a communication link between cells of native and acquired immunity. Cells respond to cytokines with specific biological changes that are dependent on the activation of new gene expression.

[0003] Studies of the mechanism by which signals are mediated from the receptor to the nucleus by the interferon cytokines have revealed the activation of latent cytoplasmic transcription factors that subsequently translocate to the nucleus (Bromberg, *BioEssays*, 2001, 23, 161-169).

[0004] The proteins of the STAT family (signal transducers and activators of transcription) are latent transcription factors that are abundantly expressed in many cell types. STATs are activated by phosphorylation on a single tyrosine in response to extracellular ligands. An active STAT dimer is formed through reciprocal interactions between the SH2 domain of one monomer and the phosphorylated tyrosine of the other monomer. The dimers accumulate in the nucleus, recognize specific DNA elements in the promoters of genes and activate transcription so that growth control and survival of normal cells in a developing or adult mammal are carefully balanced. Many of the signals that influence this balance are delivered by circulating polypeptides, whose binding to cell surface receptors governs gene-specific transcription. It has been shown that human cancer cells have lost control of these signaling mechanisms. In addition to persistent unregulated mitogenic signaling, the lack of suppressive signals is also critical in the development of cancers (Bromberg, *BioEssays*, 2001, 23, 161-169).

[0005] STAT 6 (also known as interleukin 4-STAT) was cloned and mapped to chromosome 12q13 (Leek et al., *Cytogenet. Cell Genet.*, 1997, 79, 208-209; Quelle et al., *Mol. Cell Biol.*, 1995, 15, 3336-3343). Nucleic acid sequences encoding STAT 6 are disclosed and claimed in U.S. Pat. No. 5,710,266 (McKnight and Hou, 1998).

[0006] STAT 6 is primarily expressed as a 4 kb transcript in hematopoietic cells and expressed variably in other tissues (Quelle et al., *Mol. Cell Biol.*, 1995, 15, 3336-3343). A unique truncated isoform of STAT 6 is expressed in mast cells (Sherman, *Immunol. Rev.*, 2001, 179, 48-56). Disclosed and claimed in PCT publication WO 99/10493 are nucleic acid sequences encoding variants of STAT 6 known as STAT 6b and STAT 6c as well as vectors comprising said nucleic acid sequences (Patel et al., 1999).

[0007] STAT 6 is an integral transcription factor involved in interleukin 4 and interleukin 13 signaling. Following activation of their respective receptors, interleukin 4 and interleukin 13 cause their common interleukin 4 receptor

alpha chain to become phosphorylated by JAK3 and to subsequently bind to STAT 6. STAT 6 is then phosphorylated by JAK1, homodimerizes and translocates to the nucleus where it binds interleukin 4 response elements and initiates the transcription of a number of genes including IgE (Danahay et al., *Inflamm. Res.*, 2000, 49, 692-699).

[0008] STAT 6 is involved in key pathological mechanisms in rheumatoid arthritis which operate in early and late stages of the disease (Muller-Ladner et al., *J. Immunol.*, 2000, 164, 3894-3901).

[0009] Ghilardi et al. have found that STAT 6 interacts with an isoform of the leptin receptor (OB-R) and is thus, a potential mediator of the anti-obesity effects of leptin (Ghilardi et al., *Proc. Natl. Acad. Sci. U. S. A.*, 1996, 93, 6231-6235).

[0010] STAT 6 knockout mice are viable and develop normally with the exception that interleukin 4 functions are eliminated (Ihle, *Curr. Opin. Cell Biol.*, 2001, 13, 211-217). Additionally, STAT 6 knockout mice fail to develop antigen-induced airway hyper-reactivity in a model of airway inflammation (Kuperman et al., *J. Exp. Med.*, 1998, 187, 939-948).

[0011] Inhibition of STAT 6 is expected to attenuate the allergic response and thus, represents an attractive target for drug discovery strategies (Hill et al., *Am. J. Respir. Cell Mol. Biol.*, 1999, 21, 728-737).

[0012] Small molecule inhibitors of STAT 6 are disclosed and claimed in PCT publication WO 00/27802 and Japanese Patent JP 2000229959 (Eyer mann et al., 2000; Inoue et al., *PCT*, 2000, Abstract only). Disclosed and claimed in U.S. Pat. No. 6,207,391 are methods for screening modulators of STAT 6 binding to a STAT 6 receptor (Wu and McKinney, 2001).

[0013] Wang et al. have demonstrated targeted disruption of STAT 6 DNA-binding activity by a phosphorothioate cis-element decoy oligonucleotide (Wang et al., *Blood*, 2000, 95, 1249-1257).

[0014] Hill et al. have used a series of homologous human and murine antisense oligonucleotides targeting STAT 6 to interrupt interleukin 4 and interleukin 13 signaling and attenuate germline C-epsilon transcription in vitro (Hill et al., *Am. J. Respir. Cell Mol. Biol.*, 1999, 21, 728-737). Subsequently, the in vitro and in vivo pharmacology of three of the antisense oligonucleotides used in the latter study was investigated. Although the oligonucleotides downregulated STAT 6 mRNA, their action was not sufficient to influence alterations in mRNA levels (Danahay et al., *Inflamm. Res.*, 2000, 49, 692-699).

[0015] Currently, there are no known therapeutic agents that effectively inhibit the synthesis of STAT 6. Consequently, there remains a long felt need for additional agents capable of effectively inhibiting STAT 6 function.

[0016] Antisense technology is emerging as an effective means for reducing the expression of specific gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of STAT 6 expression.

[0017] The present invention provides compositions and methods for modulating STAT 6 expression.

## SUMMARY OF THE INVENTION

[0018] The present invention is directed to compounds, especially nucleic acid and nucleic acid-like oligomers, which are targeted to a nucleic acid encoding STAT 6, and which modulate the expression of STAT 6. Pharmaceutical and other compositions comprising the compounds of the invention are also provided. Further provided are methods of screening for modulators of STAT 6 and methods of modulating the expression of STAT 6 in cells, tissues or animals comprising contacting said cells, tissues or animals with one or more of the compounds or compositions of the invention. Methods of treating an animal, particularly a human, suspected of having or being prone to a disease or condition associated with expression of STAT 6 are also set forth herein. Such methods comprise administering a therapeutically or prophylactically effective amount of one or more of the compounds or compositions of the invention to the person in need of treatment.

## DETAILED DESCRIPTION OF THE INVENTION

## [0019] A. Overview of the Invention

[0020] The present invention employs compounds, preferably oligonucleotides and similar species for use in modulating the function or effect of nucleic acid molecules encoding STAT 6. This is accomplished by providing oligonucleotides which specifically hybridize with one or more nucleic acid molecules encoding STAT 6. As used herein, the terms "target nucleic acid" and "nucleic acid molecule encoding STAT 6" have been used for convenience to encompass DNA encoding STAT 6, RNA (including pre-mRNA and mRNA or portions thereof) transcribed from such DNA, and also cDNA derived from such RNA. The hybridization of a compound of this invention with its target nucleic acid is generally referred to as "antisense". Consequently, the preferred mechanism believed to be included in the practice of some preferred embodiments of the invention is referred to herein as "antisense inhibition." Such antisense inhibition is typically based upon hydrogen bonding-based hybridization of oligonucleotide strands or segments such that at least one strand or segment is cleaved, degraded, or otherwise rendered inoperable. In this regard, it is presently preferred to target specific nucleic acid molecules and their functions for such antisense inhibition.

[0021] The functions of DNA to be interfered with can include replication and transcription. Replication and transcription, for example, can be from an endogenous cellular template, a vector, a plasmid construct or otherwise. The functions of RNA to be interfered with can include functions such as translocation of the RNA to a site of protein translation, translocation of the RNA to sites within the cell which are distant from the site of RNA synthesis, translation of protein from the RNA, splicing of the RNA to yield one or more RNA species, and catalytic activity or complex formation involving the RNA which may be engaged in or facilitated by the RNA. One preferred result of such interference with target nucleic acid function is modulation of the expression of STAT 6. In the context of the present invention, "modulation" and "modulation of expression" mean either an increase (stimulation) or a decrease (inhibition) in the amount or levels of a nucleic acid molecule encoding the gene, e.g., DNA or RNA. Inhibition is often the preferred form of modulation of expression and mRNA is often a preferred target nucleic acid.

[0022] In the context of this invention, "hybridization" means the pairing of complementary strands of oligomeric compounds. In the present invention, the preferred mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleobases) of the strands of oligomeric compounds. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds. Hybridization can occur, under varying circumstances.

[0023] An antisense compound is specifically hybridizable when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and under conditions in which assays are performed in the case of in vitro assays.

[0024] In the present invention the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a compound of the invention will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances and in the context of this invention, "stringent conditions" under which oligomeric compounds hybridize to a target sequence are determined by the nature and composition of the oligomeric compounds and the assays in which they are being investigated.

[0025] "Complementary," as used herein, refers to the capacity for precise pairing between two nucleobases of an oligomeric compound. For example, if a nucleobase at a certain position of an oligonucleotide (an oligomeric compound), is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, said target nucleic acid being a DNA, RNA, or oligonucleotide molecule, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be a complementary position. The oligonucleotide and the further DNA, RNA, or oligonucleotide molecule are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleobases which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleobases such that stable and specific binding occurs between the oligonucleotide and a target nucleic acid.

[0026] It is understood in the art that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, an oligonucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure or hairpin structure). It is preferred that the antisense compounds of the present invention comprise at least 70% sequence complementarity to a target region within the target nucleic acid, more preferably that they comprise 90% sequence complementarity and even more preferably com-

prise 95% sequence complementarity to the target region within the target nucleic acid sequence to which they are targeted. For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul et al., *J. Mol. Biol.*, 1990, 215, 403-410; Zhang and Madden, *Genome Res.*, 1997, 7, 649-656).

#### [0027] B. Compounds of the Invention

[0028] According to the present invention, compounds include antisense oligomeric compounds, antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges or loops. Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect modification of the target nucleic acid. One non-limiting example of such an enzyme is RNase H, a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. It is known in the art that single-stranded antisense compounds which are "DNA-like" elicit RNase H. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide-mediated inhibition of gene expression. Similar roles have been postulated for other ribonucleases such as those in the RNase III and ribonuclease L family of enzymes.

[0029] While the preferred form of antisense compound is a single-stranded antisense oligonucleotide, in many species the introduction of double-stranded structures, such as double-stranded RNA (dsRNA) molecules, has been shown to induce potent and specific antisense-mediated reduction of the function of a gene or its associated gene products. This phenomenon occurs in both plants and animals and is believed to have an evolutionary connection to viral defense and transposon silencing.

[0030] The first evidence that dsRNA could lead to gene silencing in animals came in 1995 from work in the nematode, *Caenorhabditis elegans* (Guo and Kempheus, *Cell*, 1995, 81, 611-620). Montgomery et al. have shown that the primary interference effects of dsRNA are posttranscriptional (Montgomery et al., *Proc. Natl. Acad. Sci. USA*, 1998, 95, 15502-15507). The posttranscriptional antisense mechanism defined in *Caenorhabditis elegans* resulting from exposure to double-stranded RNA (dsRNA) has since been

designated RNA interference (RNAi). This term has been generalized to mean antisense-mediated gene silencing involving the introduction of dsRNA leading to the sequence-specific reduction of endogenous targeted mRNA levels (Fire et al., *Nature*, 1998, 391, 806-811). Recently, it has been shown that it is, in fact, the single-stranded RNA oligomers of antisense polarity of the dsRNAs which are the potent inducers of RNAi (Tijsterman et al., *Science*, 2002, 295, 694-697).

[0031] In the context of this invention, the term "oligomeric compound" refers to a polymer or oligomer comprising a plurality of monomeric units. In the context of this invention, the term oligonucleotide refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics, chimeras, analogs and homologs thereof. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for a target nucleic acid and increased stability in the presence of nucleases.

[0032] While oligonucleotides are a preferred form of the compounds of this invention, the present invention comprehends other families of compounds as well, including but not limited to oligonucleotide analogs and mimetics such as those described herein.

[0033] The compounds in accordance with this invention preferably comprise from about 8 to about 80 nucleobases (i.e. from about 8 to about 80 linked nucleosides). One of ordinary skill in the art will appreciate that the invention embodies compounds of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleobases in length.

[0034] In one preferred embodiment, the compounds of the invention are 12 to 50 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies compounds of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleobases in length.

[0035] In another preferred embodiment, the compounds of the invention are 15 to 30 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies compounds of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length.

[0036] Particularly preferred compounds are oligonucleotides from about 12 to about 50 nucleobases, even more preferably those comprising from about 15 to about 30 nucleobases.

[0037] Antisense compounds 8-80 nucleobases in length comprising a stretch of at least eight (8) consecutive nucleobases selected from within the illustrative antisense compounds are considered to be suitable antisense compounds as well.

**[0038]** Exemplary preferred antisense compounds include oligonucleotide sequences that comprise at least the 8 consecutive nucleobases from the 5'-terminus of one of the illustrative preferred antisense compounds (the remaining nucleobases being a consecutive stretch of the same oligonucleotide beginning immediately upstream of the 5'-terminus of the antisense compound which is specifically hybridizable to the target nucleic acid and continuing until the oligonucleotide contains about 8 to about 80 nucleobases). Similarly preferred antisense compounds are represented by oligonucleotide sequences that comprise at least the 8 consecutive nucleobases from the 3'-terminus of one of the illustrative preferred antisense compounds (the remaining nucleobases being a consecutive stretch of the same oligonucleotide beginning immediately downstream of the 3'-terminus of the antisense compound which is specifically hybridizable to the target nucleic acid and continuing until the oligonucleotide contains about 8 to about 80 nucleobases). One having skill in the art armed with the preferred antisense compounds illustrated herein will be able, without undue experimentation, to identify further preferred antisense compounds.

**[0039]** C. Targets of the Invention

**[0040]** "Targeting" an antisense compound to a particular nucleic acid molecule, in the context of this invention, can be a multistep process. The process usually begins with the identification of a target nucleic acid whose function is to be modulated. This target nucleic acid may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target nucleic acid encodes STAT 6.

**[0041]** The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect, e.g., modulation of expression, will result. Within the context of the present invention, the term "region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. Within regions of target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic acid. "Sites," as used in the present invention, are defined as positions within a target nucleic acid.

**[0042]** Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the

invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used in vivo to initiate translation of an mRNA transcribed from a gene encoding STAT 6, regardless of the sequence(s) of such codons. It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively).

**[0043]** The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon. Consequently, the "start codon region" (or "translation initiation codon region") and the "stop codon region" (or "translation termination codon region") are all regions which may be targeted effectively with the antisense compounds of the present invention.

**[0044]** The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Within the context of the present invention, a preferred region is the intragenic region encompassing the translation initiation or termination codon of the open reading frame (ORF) of a gene.

**[0045]** Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA (or corresponding nucleotides on the gene), and the 3' untranslated region (3' UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA (or corresponding nucleotides on the gene). The 5' cap site of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap site. It is also preferred to target the 5' cap region.

**[0046]** Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. Targeting splice sites, i.e., intron-exon junctions or exon-intron junctions, may also be particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also preferred target sites. mRNA transcripts produced via the process of splicing of two (or more) mRNAs from different gene sources are known as "fusion transcripts". It

is also known that introns can be effectively targeted using antisense compounds targeted to, for example, DNA or pre-mRNA.

[0047] It is also known in the art that alternative RNA transcripts can be produced from the same genomic region of DNA. These alternative transcripts are generally known as “variants”. More specifically, “pre-mRNA variants” are transcripts produced from the same genomic DNA that differ from other transcripts produced from the same genomic DNA in either their start or stop position and contain both intronic and exonic sequence.

[0048] Upon excision of one or more exon or intron regions, or portions thereof during splicing, pre-mRNA variants produce smaller “mRNA variants”. Consequently, mRNA variants are processed pre-mRNA variants and each unique pre-mRNA variant must always produce a unique mRNA variant as a result of splicing. These mRNA variants are also known as “alternative splice variants”. If no splicing of the pre-mRNA variant occurs then the pre-mRNA variant is identical to the mRNA variant.

[0049] It is also known in the art that variants can be produced through the use of alternative signals to start or stop transcription and that pre-mRNAs and mRNAs can possess more than one start codon or stop codon. Variants that originate from a pre-mRNA or mRNA that use alternative start codons are known as “alternative start variants” of that pre-mRNA or mRNA. Those transcripts that use an alternative stop codon are known as “alternative stop variants” of that pre-mRNA or mRNA. One specific type of alternative stop variant is the “polyA variant” in which the multiple transcripts produced result from the alternative selection of one of the “polyA stop signals” by the transcription machinery, thereby producing transcripts that terminate at unique polyA sites. Within the context of the invention, the types of variants described herein are also preferred target nucleic acids.

[0050] The locations on the target nucleic acid to which the preferred antisense compounds hybridize are hereinbelow referred to as “preferred target segments.” As used herein the term “preferred target segment” is defined as at least an 8-nucleobase portion of a target region to which an active antisense compound is targeted. While not wishing to be bound by theory, it is presently believed that these target segments represent portions of the target nucleic acid which are accessible for hybridization.

[0051] While the specific sequences of certain preferred target segments are set forth herein, one of skill in the art will recognize that these serve to illustrate and describe particular embodiments within the scope of the present invention. Additional preferred target segments may be identified by one having ordinary skill.

[0052] Target segments 8-80 nucleobases in length comprising a stretch of at least eight (8) consecutive nucleobases selected from within the illustrative preferred target segments are considered to be suitable for targeting as well.

[0053] Target segments can include DNA or RNA sequences that comprise at least the 8 consecutive nucleobases from the 5'-terminus of one of the illustrative preferred target segments (the remaining nucleobases being a consecutive stretch of the same DNA or RNA beginning immediately upstream of the 5'-terminus of the target segment and

continuing until the DNA or RNA contains about 8 to about 80 nucleobases). Similarly preferred target segments are represented by DNA or RNA sequences that comprise at least the 8 consecutive nucleobases from the 3'-terminus of one of the illustrative preferred target segments (the remaining nucleobases being a consecutive stretch of the same DNA or RNA beginning immediately downstream of the 3'-terminus of the target segment and continuing until the DNA or RNA contains about 8 to about 80 nucleobases). One having skill in the art armed with the preferred target segments illustrated herein will be able, without undue experimentation, to identify further preferred target segments.

[0054] Once one or more target regions, segments or sites have been identified, antisense compounds are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

#### [0055] D. Screening and Target Validation

[0056] In a further embodiment, the “preferred target segments” identified herein may be employed in a screen for additional compounds that modulate the expression of STAT 6. “Modulators” are those compounds that decrease or increase the expression of a nucleic acid molecule encoding STAT 6 and which comprise at least an 8-nucleobase portion which is complementary to a preferred target segment. The screening method comprises the steps of contacting a preferred target segment of a nucleic acid molecule encoding STAT 6 with one or more candidate modulators, and selecting for one or more candidate modulators which decrease or increase the expression of a nucleic acid molecule encoding STAT 6. Once it is shown that the candidate modulator or modulators are capable of modulating (e.g. either decreasing or increasing) the expression of a nucleic acid molecule encoding STAT 6, the modulator may then be employed in further investigative studies of the function of STAT 6, or for use as a research, diagnostic, or therapeutic agent in accordance with the present invention.

[0057] The preferred target segments of the present invention may be also be combined with their respective complementary antisense compounds of the present invention to form stabilized double-stranded (duplexed) oligonucleotides.

[0058] Such double stranded oligonucleotide moieties have been shown in the art to modulate target expression and regulate translation as well as RNA processing via an antisense mechanism. Moreover, the double-stranded moieties may be subject to chemical modifications (Fire et al., *Nature*, 1998, 391, 806-811; Timmons and Fire, *Nature* 1998, 395, 854; Timmons et al., *Gene*, 2001, 263, 103-112; Tabara et al., *Science*, 1998, 282, 430-431; Montgomery et al., *Proc. Natl. Acad. Sci. USA*, 1998, 95, 15502-15507; Tuschl et al., *Genes Dev.*, 1999, 13, 3191-3197; Elbashir et al., *Nature*, 2001, 411, 494-498; Elbashir et al., *Genes Dev.* 2001, 15, 188-200). For example, such double-stranded moieties have been shown to inhibit the target by the classical hybridization of antisense strand of the duplex to the target, thereby triggering enzymatic degradation of the target (Tijsterman et al., *Science*, 2002, 295, 694-697).

[0059] The compounds of the present invention can also be applied in the areas of drug discovery and target valida-

tion. The present invention comprehends the use of the compounds and preferred target segments identified herein in drug discovery efforts to elucidate relationships that exist between STAT 6 and a disease state, phenotype, or condition. These methods include detecting or modulating STAT 6 comprising contacting a sample, tissue, cell, or organism with the compounds of the present invention, measuring the nucleic acid or protein level of STAT 6 and/or a related phenotypic or chemical endpoint at some time after treatment, and optionally comparing the measured value to a non-treated sample or sample treated with a further compound of the invention. These methods can also be performed in parallel or in combination with other experiments to determine the function of unknown genes for the process of target validation or to determine the validity of a particular gene product as a target for treatment or prevention of a particular disease, condition, or phenotype.

**[0060]** E. Kits, Research Reagents, Diagnostics, and Therapeutics

**[0061]** The compounds of the present invention can be utilized for diagnostics, therapeutics, prophylaxis and as research reagents and kits. Furthermore, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes or to distinguish between functions of various members of a biological pathway.

**[0062]** For use in kits and diagnostics, the compounds of the present invention, either alone or in combination with other compounds or therapeutics, can be used as tools in differential and/or combinatorial analyses to elucidate expression patterns of a portion or the entire complement of genes expressed within cells and tissues.

**[0063]** As one nonlimiting example, expression patterns within cells or tissues treated with one or more antisense compounds are compared to control cells or tissues not treated with antisense compounds and the patterns produced are analyzed for differential levels of gene expression as they pertain, for example, to disease association, signaling pathway, cellular localization, expression level, size, structure or function of the genes examined. These analyses can be performed on stimulated or unstimulated cells and in the presence or absence of other compounds which affect expression patterns.

**[0064]** Examples of methods of, gene expression analysis known in the art include DNA arrays or microarrays (Brazma and Vilo, *FEBS Lett.*, 2000, 480, 17-24; Celis, et al., *FEBS Lett.*, 2000, 480, 2-16), SAGE (serial analysis of gene expression)(Madden, et al., *Drug Discov. Today*, 2000, 5, 415-425), READS (restriction enzyme amplification of digested cDNAs) (Prashar and Weissman, *Methods Enzymol.*, 1999, 303, 258-72), TOGA (total gene expression analysis) (Sutcliffe, et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 1976-81), protein arrays and proteomics (Celis, et al., *FEBS Lett.*, 2000, 480, 2-16; Jungblut, et al., *Electrophoresis*, 1999, 20, 2100-10), expressed sequence tag (EST) sequencing (Celis, et al., *FEBS Lett.*, 2000, 480, 2-16; Larsson, et al., *J. Biotechnol.*, 2000, 80, 143-57), subtractive RNA fingerprinting (SuRF) (Fuchs, et al., *Anal. Biochem.*, 2000, 286, 91-98; Larson, et al., *Cytometry*, 2000, 41, 203-208), subtractive cloning, differential display (DD) (Jurecic and Belmont, *Curr. Opin. Microbiol.*, 2000, 3,

316-21), comparative genomic hybridization (Carulli, et al., *J. Cell Biochem. Suppl.*, 1998, 31, 286-96), FISH (fluorescent in situ hybridization) techniques (Going and Gusterson, *Eur. J. Cancer*, 1999, 35, 1895-904) and mass spectrometry methods (To, *Comb. Chem. High Throughput Screen*, 2000, 3, 235-41).

**[0065]** The compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding STAT 6. For example, oligonucleotides that are shown to hybridize with such efficiency and under such conditions as disclosed herein as to be effective STAT 6 inhibitors will also be effective primers or probes under conditions favoring gene amplification or detection, respectively. These primers and probes are useful in methods requiring the specific detection of nucleic acid molecules encoding STAT 6 and in the amplification of said nucleic acid molecules for detection or for use in further studies of STAT 6. Hybridization of the antisense oligonucleotides, particularly the primers and probes, of the invention with a nucleic acid encoding STAT 6 can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabelling of the oligonucleotide or any other suitable detection means. Kits using such detection means for detecting the level of STAT 6 in a sample may also be prepared.

**[0066]** The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense compounds have been employed as therapeutic moieties in the treatment of disease states in animals, including humans. Antisense oligonucleotide drugs, including ribozymes, have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that antisense compounds can be useful therapeutic modalities that can be configured to be useful in treatment regimes for the treatment of cells, tissues and animals, especially humans.

**[0067]** For therapeutics, an animal, preferably a human, suspected of having a disease or disorder which can be treated by modulating the expression of STAT 6 is treated by administering antisense compounds in accordance with this invention. For example, in one non-limiting embodiment, the methods comprise the step of administering to the animal in need of treatment, a therapeutically effective amount of a STAT 6 inhibitor. The STAT 6 inhibitors of the present invention effectively inhibit the activity of the STAT 6 protein or inhibit the expression of the STAT 6 protein. In one embodiment, the activity or expression of STAT 6 in an animal is inhibited by about 10%. Preferably, the activity or expression of STAT 6 in an animal is inhibited by about 30%. More preferably, the activity or expression of STAT 6 in an animal is inhibited by 50% or more.

**[0068]** For example, the reduction of the expression of STAT 6 may be measured in serum, adipose tissue, liver or any other body fluid, tissue or organ of the animal. Preferably, the cells contained within said fluids, tissues or organs being analyzed contain a nucleic acid molecule encoding STAT 6 protein and/or the STAT 6 protein itself.

**[0069]** The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of a compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the compounds and methods of the invention may also be useful prophylactically.

**[0070]** F. Modifications

**[0071]** As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn, the respective ends of this linear polymeric compound can be further joined to form a circular compound, however, linear compounds are generally preferred. In addition, linear compounds may have internal nucleobase complementarity and may therefore fold in a manner as to produce a fully or partially double-stranded compound. Within oligonucleotides, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

**[0072]** Modified Internucleoside Linkages (Backbones)

**[0073]** Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

**[0074]** Preferred modified oligonucleotide backbones containing a phosphorus atom therein include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thiono-phosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Preferred oligonucleotides having inverted polarity comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage i.e. a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts, mixed salts and free acid forms are also included.

**[0075]** Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,194,599; 5,565,555;

5,527,899; 5,721,218; 5,672,697 and 5,625,050, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

**[0076]** Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH<sub>2</sub> component parts.

**[0077]** Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; 5,792,608; 5,646,269 and 5,677,439, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

**[0078]** Modified Sugar and Internucleoside Linkages-Mimetics

**[0079]** In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage (i.e. the backbone), of the nucleotide units are replaced with novel groups. The nucleobase units are maintained for hybridization with an appropriate target nucleic acid. One such compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., *Science*, 1991, 254, 1497-1500.

**[0080]** Preferred embodiments of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular —CH<sub>2</sub>—NH—O—CH<sub>2</sub>—, —CH<sub>2</sub>—N(CH<sub>3</sub>)—O—CH<sub>2</sub>— [known as a methylene (methylimino) or MMI backbone], —CH<sub>2</sub>—O—N(CH<sub>3</sub>)—CH<sub>2</sub>—, —CH<sub>2</sub>—N(CH<sub>3</sub>)—N(CH<sub>3</sub>)—CH<sub>2</sub>— and —O—N(CH<sub>3</sub>)—CH<sub>2</sub>—CH<sub>2</sub>— [wherein the native phosphodiester backbone is represented as —O—P—O—CH<sub>2</sub>—] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

**[0081] Modified Sugars**

**[0082]** Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O—, S—, or N-alkyl; O—, S—, or N-alkenyl; O—, S— or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C<sub>1</sub> to C<sub>10</sub> alkyl or C<sub>2</sub> to C<sub>10</sub> alkenyl and alkynyl. Particularly preferred are O[(CH<sub>2</sub>)<sub>n</sub>O]<sub>m</sub>CH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub>OCH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub>NH<sub>2</sub>, O(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub>ONH<sub>2</sub>, and O(CH<sub>2</sub>)<sub>n</sub>ON[(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>]<sub>2</sub>, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C<sub>1</sub> to C<sub>10</sub> lower alkyl, substituted lower alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH<sub>3</sub>, OCN, Cl, Br, CN, CF<sub>3</sub>, OCF<sub>3</sub>, SOCH<sub>3</sub>, SO<sub>2</sub>CH<sub>3</sub>, ONO<sub>2</sub>, NO<sub>2</sub>, N<sub>3</sub>, NH<sub>2</sub>, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O—CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, also known as 2'-O—(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta*, 1995, 78, 486-504) i.e., an alkoxy-alkoxy group. A further preferred modification includes 2'-dimethylaminoethoxy, i.e., a O(CH<sub>2</sub>)<sub>2</sub>ON(CH<sub>3</sub>)<sub>2</sub> group, also known as 2'-DMAOE, as described in examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethyl-amino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O—CH<sub>2</sub>—O—CH<sub>2</sub>—N(CH<sub>3</sub>)<sub>2</sub>, also described in examples hereinbelow.

**[0083]** Other preferred modifications include 2'-methoxy (2'-O—CH<sub>3</sub>), 2'-aminopropoxy (2'-OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 2'-allyl (2'-CH<sub>2</sub>—CH=CH<sub>2</sub>), 2'-O-allyl (2'-O—CH<sub>2</sub>—CH=CH<sub>2</sub>) and 2'-fluoro (2'-F). The 2'-modification may be in the arabino (up) position or ribo (down) position. A preferred 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

**[0084]** A further preferred modification of the sugar includes Locked Nucleic Acids (LNAs) in which the 2'-hydroxyl group is linked to the 3' or 4' carbon atom of the sugar ring, thereby forming a bicyclic sugar moiety. The linkage is preferably a methylene (—CH<sub>2</sub>—)<sub>n</sub> group bridging the 2' oxygen atom and the 4' carbon atom wherein n is 1 or 2. LNAs and preparation thereof are described in WO 98/39352 and WO 99/14226.

**[0085] Natural and Modified Nucleobases**

**[0086]** Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (—C≡C—CH<sub>3</sub>) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine (1H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridoindole cytidine (H-pyrido[3', 2':4,5]pyrrolo[2,3-d]pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., *Angewandte Chemie*, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y. S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S. T. and Lebleu, B. ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

**[0087]** Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121; 5,596,091; 5,614,617; 5,645,985; 5,830,653; 5,763,588; 6,005,096; and 5,681,941, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, which is commonly owned with the instant application and also herein incorporated by reference.

**[0088]** Conjugates

**[0089]** Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. These moieties or conjugates can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl groups. Conjugate groups of the invention include intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugate groups include cholesterol, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve uptake, enhance resistance to degradation, and/or strengthen sequence-specific hybridization with the target nucleic acid. Groups that enhance the pharmacokinetic properties, in the context of this invention, include groups that improve uptake, distribution, metabolism or excretion of the compounds of the present invention. Representative conjugate groups are disclosed in International Patent Application PCT/US92/09196, filed Oct. 23, 1992, and U.S. Pat. No. 6,287,860, the entire disclosure of which are incorporated herein by reference. Conjugate moieties include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g., hexyl-S-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety. Oligonucleotides of the invention may also be conjugated to active drug substances, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fenbufen, ketoprofen, (S)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, flufenamic acid, folic acid, a benzothiadiazide, chlorothiazide, a diazepam, indomethacin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic. Oligonucleotide-drug conjugates and their preparation are described in U.S. patent application Ser. No. 09/334,130 (filed Jun. 15, 1999) which is incorporated herein by reference in its entirety.

**[0090]** Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference.

**[0091]** Chimeric Compounds

**[0092]** It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide.

**[0093]** The present invention also includes antisense compounds which are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, increased stability and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide-mediated inhibition of gene expression. The cleavage of RNA:RNA hybrids can, in like fashion, be accomplished through the actions of endoribonucleases, such as RNaseL which cleaves both cellular and viral RNA. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

**[0094]** Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S. Pat. Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

**[0095]** G. Formulations

**[0096]** The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption-assisting formulations include, but are not limited to, U.S. Pat. Nos. 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

**[0097]** The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of

such esters, or any other compound which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the compounds of the invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

**[0098]** The term “prodrug” indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the oligonucleotides of the invention are prepared as SATE [(S-acetyl-2-thioethyl) phosphate] derivatives according to the methods disclosed in WO 93/24510 to Gosselin et al., published Dec. 9, 1993 or in WO 94/26764 and U.S. Pat. No. 5,770,713 to Imbach et al.

**[0099]** The term “pharmaceutically acceptable salts” refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. For oligonucleotides, preferred examples of pharmaceutically acceptable salts and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

**[0100]** The present invention also includes pharmaceutical compositions and formulations which include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

**[0101]** The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

**[0102]** The compositions of the present invention may be formulated into any of many possible dosage forms such as,

but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

**[0103]** Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical compositions and formulations of the present invention may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

**[0104]** Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1  $\mu\text{m}$  in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment of the present invention. Emulsions and their uses are well known in the art and are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

**[0105]** Formulations of the present invention include liposomal formulations. As used in the present invention, the term “liposome” means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes which are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

**[0106]** Liposomes also include “sterically stabilized” liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

**[0107]** The pharmaceutical formulations and compositions of the present invention may also include surfactants. The use of surfactants in drug products, formulations and in emulsions is well known in the art. Surfactants and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

**[0108]** In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly oligonucleotides. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the perme-

ability of lipophilic drugs. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants. Penetration enhancers and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

**[0109]** One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

**[0110]** Preferred formulations for topical administration include those in which the oligonucleotides of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA).

**[0111]** For topical or other administration, oligonucleotides of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, oligonucleotides may be complexed to lipids, in particular to cationic lipids. Preferred fatty acids and esters, pharmaceutically acceptable salts thereof, and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety. Topical formulations are described in detail in U.S. patent application Ser. No. 09/315,298 filed on May 20, 1999, which is incorporated herein by reference in its entirety.

**[0112]** Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitabets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which oligonucleotides of the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts and fatty acids and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety. Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. Oligonucleotides of the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. Oligonucleotide complexing agents and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety. Oral formulations for oligonucleotides and their preparation are described in detail in U.S. application Ser. No. 09/108,673 (filed Jul. 1, 1998), Ser. No. 09/315,298 (filed May 20, 1999) and Ser. No. 10/071,822, filed Feb. 8, 2002, each of which is incorporated herein by reference in their entirety.

**[0113]** Compositions and formulations for parenteral, intrathecal or intraventricular administration may include

sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

**[0114]** Certain embodiments of the invention provide pharmaceutical compositions containing one or more oligomeric compounds and one or more other chemotherapeutic agents which function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to cancer chemotherapeutic drugs such as daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycytosine, 4-hydroxypyrimidinocyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). When used with the compounds of the invention, such chemotherapeutic agents may be used individually (e.g., 5-FU and oligonucleotide), sequentially (e.g., 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (e.g., 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Combinations of antisense compounds and other non-antisense drugs are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

**[0115]** In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Alternatively, compositions of the invention may contain two or more antisense compounds targeted to different regions of the same nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more combined compounds may be used together or sequentially.

**[0116]** H. Dosing

**[0117]** The formulation of therapeutic compositions and their subsequent administration (dosing) is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on

the relative potency of individual oligonucleotides, and can generally be estimated based on EC<sub>50</sub>s found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 ug to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 ug to 100 g per kg of body weight, once or more daily, to once every 20 years.

[0118] While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same.

## EXAMPLES

### Example 1

#### [0119] Synthesis of Nucleoside Phosphoramidites

[0120] The following compounds, including amidites and their intermediates were prepared as described in U.S. Pat. No. 6,426,220 and published PCT WO 02/36743; 5'-O-Dimethoxytrityl-thymidine intermediate for 5-methyl dC amidite, 5'-O-Dimethoxytrityl-2'-deoxy-5-methylcytidine intermediate for 5-methyl-dC amidite, 5'-O-Dimethoxytrityl-2'-deoxy-N<sup>4</sup>-benzoyl-5-methylcytidine penultimate intermediate for 5-methyl dC amidite, [5'-O-(4,4'-Dimethoxytriphenylmethyl)-2'-deoxy-N<sup>4</sup>-benzoyl-5-methylcytidin-3'-O-yl]-2-cyanoethyl-N,N-diisopropylphosphoramidite (5-methyl dC amidite), 2'-Fluorodeoxyadenosine, 2'-Fluorodeoxyguanosine, 2'-Fluorouridine, 2'-Fluorodeoxycytidine, 2'-O-(2-Methoxyethyl) modified amidites, 2'-O-(2-methoxyethyl)-5-methyluridine intermediate, 5'-O-DMT-2'-O-(2-methoxyethyl)-5-methyluridine penultimate intermediate, [5'-O-(4,4'-Dimethoxytriphenylmethyl)-2'-O-(2-methoxyethyl)-5-methyluridin-3'-O-yl]-2-cyanoethyl-N,N-diisopropylphosphoramidite (MOE T amidite), 5'-O-Dimethoxytrityl-2'-O-(2-methoxyethyl)-5-methylcytidine intermediate, 5'-O-dimethoxytrityl-2'-O-(2-methoxyethyl)-N<sup>4</sup>-benzoyl-5-methyl-cytidine penultimate intermediate, [5'-O-(4,4'-Dimethoxytriphenylmethyl)-2'-O-(2-methoxyethyl)-N<sup>4</sup>-benzoyl-5-methylcytidin-3'-O-yl]-2-cyanoethyl-N,N-diisopropylphosphoramidite (MOE 5-Me-C amidite), [5'-O-(4,4'-Dimethoxytriphenylmethyl)-2'-O-(2-methoxyethyl)-N<sup>6</sup>-benzoyl-adenosin-3'-O-yl]-2-cyanoethyl-N,N-diisopropylphosphoramidite (MOE A amidite), [5'-O-(4,4'-Dimethoxytriphenylmethyl)-2'-O-(2-methoxyethyl)-N<sup>4</sup>-isobutrylguanosin-3'-O-yl]-2-cyanoethyl-N,N-diisopropylphosphoramidite (MOE G amidite), 2'-O-(Aminooxyethyl) nucleoside amidites and 2'-O-(dimethylaminoxyethyl) nucleoside amidites, 2'-(Dimethylaminoxyethoxy) nucleoside amidites, 5'-O-tert-Butyldiphenylsilyl-O<sup>2</sup>-2'-anhydro-5-methyluridine, 5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine, 2'-O-[(2-phthalimidoxy)ethyl]-5'-t-butylidiphenylsilyl-5-methyluridine, 5'-O-tert-butylidiphenylsilyl-2'-O-[(2-formadoximinoxy)ethyl]-5-methyluridine, 5'-O-tert-Butyldiphenylsilyl-2'-O-[N,N dimethylaminoxyethyl]-5-

methyluridine, 2'-O-(dimethylaminoxyethyl)-5-methyluridine, 5'-O-DMT-2'-O-(dimethylaminoxyethyl)-5-methyluridine, 5'-O-DMT-2'-O-(2-N,N-dimethylaminoxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite], 2'-(Aminooxyethoxy) nucleoside amidites, N<sup>2</sup>-isobutryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite], 2'-dimethylaminoethoxyethoxy (2'-DMAEOE) nucleoside amidites, 2'-O-[2(2-N,N-dimethylaminoethoxy)-ethyl]-5-methyl uridine, 5'-O-dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)-ethyl]-5-methyl uridine and 5'-O-Dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)-ethyl]-5-methyl uridine-3'-O-(cyanoethyl-N,N-diisopropyl)phosphoramidite.

### Example 2

#### [0121] Oligonucleotide and Oligonucleoside Synthesis

[0122] The antisense compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and alkylated derivatives.

[0123] Oligonucleotides: Unsubstituted and substituted phosphodiester (P=O) oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 394) using standard phosphoramidite chemistry with oxidation by iodine.

[0124] Phosphorothioates (P=S) are synthesized similar to phosphodiester oligonucleotides with the following exceptions: thiation was effected by utilizing a 10% w/v solution of 3,4,5-trimethyl-1,2,4-dithiolane-3-one 1,1-dioxide in acetonitrile for the oxidation of the phosphite linkages. The thiation reaction step time was increased to 180 sec and preceded by the normal capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55° C. (12-16 hr), the oligonucleotides were recovered by precipitating with >3 volumes of ethanol from a 1 M NH<sub>4</sub>OAc solution. Phosphinate oligonucleotides are prepared as described in U.S. Pat. No. 5,508,270, herein incorporated by reference.

[0125] Alkyl phosphonate oligonucleotides are prepared as described in U.S. Pat. No. 4,469,863, herein incorporated by reference.

[0126] 3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Pat. Nos. 5,610,289 or 5,625,050, herein incorporated by reference.

[0127] Phosphoramidite oligonucleotides are prepared as described in U.S. Pat. No. 5,256,775 or U.S. Pat. No. 5,366,878, herein incorporated by reference.

[0128] Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively), herein incorporated by reference.

[0129] 3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Pat. No. 5,476,925, herein incorporated by reference.

[0130] Phosphotriester oligonucleotides are prepared as described in U.S. Pat. No. 5,023,243, herein incorporated by reference.

[0131] Borano phosphate oligonucleotides are prepared as described in U.S. Pat. Nos. 5,130,302 and 5,177,198, both herein incorporated by reference.

[0132] Oligonucleosides: Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedimethylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and P=O or P=S linkages are prepared as described in U.S. Pat. Nos. 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

[0133] Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Pat. Nos. 5,264,562 and 5,264,564, herein incorporated by reference.

[0134] Ethylene oxide linked oligonucleosides are prepared as described in U.S. Pat. No. 5,223,618, herein incorporated by reference.

### Example 3

#### [0135] RNA Synthesis

[0136] In general, RNA synthesis chemistry is based on the selective incorporation of various protecting groups at strategic intermediary reactions. Although one of ordinary skill in the art will understand the use of protecting groups in organic synthesis, a useful class of protecting groups includes silyl ethers. In particular bulky silyl ethers are used to protect the 5'-hydroxyl in combination with an acid-labile orthoester protecting group on the 2'-hydroxyl. This set of protecting groups is then used with standard solid-phase synthesis technology. It is important to lastly remove the acid labile orthoester protecting group after all other synthetic steps. Moreover, the early use of the silyl protecting groups during synthesis ensures facile removal when desired, without undesired deprotection of 2' hydroxyl.

[0137] Following this procedure for the sequential protection of the 5'-hydroxyl in combination with protection of the 2'-hydroxyl by protecting groups that are differentially removed and are differentially chemically labile, RNA oligonucleotides were synthesized.

[0138] RNA oligonucleotides are synthesized in a step-wise fashion. Each nucleotide is added sequentially (3'- to 5'-direction) to a solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are added, coupling the second base onto the 5'-end of the first nucleoside. The support is washed and any unreacted 5'-hydroxyl groups are capped with acetic anhydride to yield 5'-acetyl moieties. The linkage is then oxidized to the more stable and

ultimately desired P(V) linkage. At the end of the nucleotide addition cycle, the 5'-silyl group is cleaved with fluoride. The cycle is repeated for each subsequent nucleotide.

[0139] Following synthesis, the methyl protecting groups on the phosphates are cleaved in 30 minutes utilizing 1 M disodium-2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate ( $S_2Na_2$ ) in DMF. The deprotection solution is washed from the solid support-bound oligonucleotide using water. The support is then treated with 40% methylamine in water for 10 minutes at 55° C. This releases the RNA oligonucleotides into solution, deprotects the exocyclic amines, and modifies the 2'-groups. The oligonucleotides can be analyzed by anion exchange HPLC at this stage.

[0140] The 2'-orthoester groups are the last protecting groups to be removed. The ethylene glycol monoacetate orthoester protecting group developed by Dharmacon Research, Inc. (Lafayette, Colo.), is one example of a useful orthoester protecting group which, has the following important properties. It is stable to the conditions of nucleoside phosphoramidite synthesis and oligonucleotide synthesis. However, after oligonucleotide synthesis the oligonucleotide is treated with methylamine which not only cleaves the oligonucleotide from the solid support but also removes the acetyl groups from the orthoesters. The resulting 2-ethyl-hydroxyl substituents on the orthoester are less electron withdrawing than the acetylated precursor. As a result, the modified orthoester becomes more labile to acid-catalyzed hydrolysis. Specifically, the rate of cleavage is approximately 10 times faster after the acetyl groups are removed. Therefore, this orthoester possesses sufficient stability in order to be compatible with oligonucleotide synthesis and yet, when subsequently modified, permits deprotection to be carried out under relatively mild aqueous conditions compatible with the final RNA oligonucleotide product.

[0141] Additionally, methods of RNA synthesis are well known in the art (Scaringe, S. A. Ph.D. Thesis, University of Colorado, 1996; Scaringe, S. A., et al., *J. Am. Chem. Soc.*, 1998, 120, 11820-11821; Matteucci, M. D. and Caruthers, M. H. *J. Am. Chem. Soc.*, 1981, 103, 3185-3191; Beaucage, S. L. and Caruthers, M. H. *Tetrahedron Lett.*, 1981, 22, 1859-1862; Dahl, B. J., et al., *Acta Chem. Scand.*, 1990, 44, 639-641; Reddy, M. P., et al., *Tetrahedron Lett.*, 1994, 25, 4311-4314; Wincott, F. et al., *Nucleic Acids Res.*, 1995, 23, 2677-2684; Griffin, B. E., et al., *Tetrahedron*, 1967, 23, 2301-2313; Griffin, B. E., et al., *Tetrahedron*, 1967, 23, 2315-2331).

[0142] RNA antisense compounds (RNA oligonucleotides) of the present invention can be synthesized by the methods herein or purchased from Dharmacon Research, Inc (Lafayette, Colo.). Once synthesized, complementary RNA antisense compounds can then be annealed by methods known in the art to form double stranded (duplexed) antisense compounds. For example, duplexes can be formed by combining 30  $\mu$ l of each of the complementary strands of RNA oligonucleotides (50  $\mu$ M RNA oligonucleotide solution) and 15  $\mu$ l of 5 $\times$  annealing buffer (100 mM potassium acetate, 30 mM HEPES-KOH pH 7.4, 2 mM magnesium acetate) followed by heating for 1 minute at 90° C., then 1 hour at 37° C. The resulting duplexed antisense compounds can be used in kits, assays, screens, or other methods to investigate the role of a target nucleic acid.

## Example 4

**[0143]** Synthesis of Chimeric Oligonucleotides

**[0144]** Chimeric oligonucleotides, oligonucleosides or mixed oligonucleotides/oligonucleosides of the invention can be of several different types. These include a first type wherein the "gap" segment of linked nucleosides is positioned between 5' and 3' "wing" segments of linked nucleosides and a second "open end" type wherein the "gap" segment is located at either the 3' or the 5' terminus of the oligomeric compound. Oligonucleotides of the first type are also known in the art as "gapmers" or gapped oligonucleotides. Oligonucleotides of the second type are also known in the art as "hemimers" or "wingmers".

[2'-O-Me]—[2'-deoxy]—[2'-O-Me] Chimeric Phosphorothioate Oligonucleotides

**[0145]** Chimeric oligonucleotides having 2'-O-alkyl phosphorothioate and 2'-deoxy phosphorothioate oligonucleotide segments are synthesized using an Applied Biosystems automated DNA synthesizer Model 394, as above. Oligonucleotides are synthesized using the automated synthesizer and 2'-deoxy-5'-dimethoxytrityl-3'-O-phosphoramidite for the DNA portion and 5'-dimethoxytrityl-2'-O-methyl-3'-O-phosphoramidite for 5' and 3' wings. The standard synthesis cycle is modified by incorporating coupling steps with increased reaction times for the 5'-dimethoxytrityl-2'-O-methyl-3'-O-phosphoramidite. The fully protected oligonucleotide is cleaved from the support and deprotected in concentrated ammonia (NH<sub>4</sub>OH) for 12-16 hr at 55° C. The deprotected oligo is then recovered by an appropriate method (precipitation, column chromatography, volume reduced in vacuo and analyzed spectrophotometrically for yield and for purity by capillary electrophoresis and by mass spectrometry.

[2'-O-(2-Methoxyethyl)]—[2'-deoxy]—[2'-O-(Methoxyethyl)] Chimeric Phosphorothioate Oligonucleotides

**[0146]** [2'-O-(2-methoxyethyl)]—[2'-deoxy]—[2'-O-(methoxyethyl)] chimeric phosphorothioate oligonucleotides were prepared as per the procedure above for the 2'-O-methyl chimeric oligonucleotide, with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites.

[2'-O-(2-methoxyethyl)Phosphodiester]—[2'-deoxy Phosphorothioate]—[2'-O-(2-Methoxyethyl)Phosphodiester] Chimeric Oligonucleotides

**[0147]** [2'-O-(2-methoxyethyl phosphodiester)]—[2'-deoxy phosphorothioate]—[2'-O-(methoxyethyl)phosphodiester] chimeric oligonucleotides are prepared as per the above procedure for the 2'-O-methyl chimeric oligonucleotide with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites, oxidation with iodine to generate the phosphodiester internucleotide linkages within the wing portions of the chimeric structures and sulfuration utilizing 3,3',5,5'-tetrabenzodithiophene-2,2'-dioxide (Beaucage Reagent) to generate the phosphorothioate internucleotide linkages for the center gap.

**[0148]** Other chimeric oligonucleotides, chimeric oligonucleosides and mixed chimeric oligonucleotides/oligonucleosides are synthesized according to U.S. Pat. No. 5,623,065, herein incorporated by reference.

## Example 5

**[0149]** Design and Screening of Duplexed Antisense Compounds Targeting STAT 6

**[0150]** In accordance with the present invention, a series of nucleic acid duplexes comprising the antisense compounds of the present invention and their complements can be designed to target STAT 6. The nucleobase sequence of the antisense strand of the duplex comprises at least a portion of an oligonucleotide in Table 1. The ends of the strands may be modified by the addition of one or more natural or modified nucleobases to form an overhang. The sense strand of the dsRNA is then designed and synthesized as the complement of the antisense strand and may also contain modifications or additions to either terminus. For example, in one embodiment, both strands of the dsRNA duplex would be complementary over the central nucleobases, each having overhangs at one or both termini.

**[0151]** For example, a duplex comprising an antisense strand having the sequence CGAGAGGCGGACGG-GACCG and having a two-nucleobase overhang of deoxythymidine(dT) would have the following structure:

|                       |                  |
|-----------------------|------------------|
| cgagagggcggacgggacgTT | Antisense Strand |
|                       |                  |
| TTgtctctccgctgcctggc  | Complement       |

**[0152]** RNA strands of the duplex can be synthesized by methods disclosed herein or purchased from Dharmacon Research Inc., (Lafayette, Colo.). Once synthesized, the complementary strands are annealed. The single strands are aliquoted and diluted to a concentration of 50 uM. Once diluted, 30 uL of each strand is combined with 15 uL of a 5x solution of annealing buffer. The final concentration of said buffer is 100 mM potassium acetate, 30 mM HEPES-KOH pH 7.4, and 2 mM magnesium acetate. The final volume is 75 uL. This solution is incubated for 1 minute at 90° C. and then centrifuged for 15 seconds. The tube is allowed to sit for 1 hour at 37° C at which time the dsRNA duplexes are used in experimentation. The final concentration of the dsRNA duplex is 20 uM. This solution can be stored frozen (-20° C.) and freeze-thawed up to 5 times.

**[0153]** Once prepared, the duplexed antisense compounds are evaluated for their ability to modulate STAT 6 expression.

**[0154]** When cells reached 80% confluency, they are treated with duplexed antisense compounds of the invention. For cells grown in 96-well plates, wells are washed once with 200 uL OPTI-MEM-1 reduced-serum medium (Gibco BRL) and then treated with 130 uL of OPTI-MEM-1 containing 12 ug/mL LIPOFECTIN (Gibco BRL) and the desired duplex antisense compound at a final concentration of 200 nM. After 5 hours of treatment, the medium is replaced with fresh medium. Cells are harvested 16 hours after treatment, at which time RNA is isolated and target reduction measured by RT-PCR.

## Example 6

**[0155]** Oligonucleotide Isolation

**[0156]** After cleavage from the controlled pore glass solid support and deblocking in concentrated ammonium hydrox-

ide at 55° C. for 12-16 hours, the oligonucleotides or oligonucleosides are recovered by precipitation out of 1 M NH<sub>4</sub>OAc with >3 volumes of ethanol. Synthesized oligonucleotides were analyzed by electrospray mass spectroscopy (molecular weight determination) and by capillary gel electrophoresis and judged to be at least 70% full length material. The relative amounts of phosphorothioate and phosphodiester linkages obtained in the synthesis was determined by the ratio of correct molecular weight relative to the -16 amu product ( $\pm 32 \pm 48$ ). For some studies oligonucleotides were purified by HPLC, as described by Chiang et al., *J. Biol. Chem.* 1991, 266, 18162-18171. Results obtained with HPLC-purified material were similar to those obtained with non-HPLC purified material.

#### Example 7

**[0157]** Oligonucleotide Synthesis—96 Well Plate Format

**[0158]** Oligonucleotides were synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer capable of assembling 96 sequences simultaneously in a 96-well format. Phosphodiester internucleotide linkages were afforded by oxidation with aqueous iodine. Phosphorothioate internucleotide linkages were generated by sulfuration utilizing 3,4-dihydro-2H-benzothiole-3-one 1,1-dioxide (Beaucage Reagent) in anhydrous acetonitrile. Standard base-protected beta-cyanoethyl-diisopropyl phosphoramidites were purchased from commercial vendors (e.g. PE-Applied Biosystems, Foster City, Calif., or Pharmacia, Piscataway, N.J.). Non-standard nucleosides are synthesized as per standard or patented methods. They are utilized as base protected beta-cyanoethyl-diisopropyl phosphoramidites.

**[0159]** Oligonucleotides were cleaved from support and deprotected with concentrated NH<sub>4</sub>OH at elevated temperature (55-60° C.) for 12-16 hours and the released product then dried in vacuo. The dried product was then re-suspended in sterile water to afford a master plate from which all analytical and test plate samples are then diluted utilizing robotic pipettors.

#### Example 8

**[0160]** Oligonucleotide Analysis—96-Well Plate Format

**[0161]** The concentration of oligonucleotide in each well was assessed by dilution of samples and UV absorption spectroscopy. The full-length integrity of the individual products was evaluated by capillary electrophoresis (CE) in either the 96-well format (Beckman P/ACE™ MDQ) or, for individually prepared samples, on a commercial CE apparatus (e.g., Beckman P/ACE™ 5000, ABI 270). Base and backbone composition was confirmed by mass analysis of the compounds utilizing electrospray-mass spectroscopy. All assay test plates were diluted from the master plate using single and multi-channel robotic pipettors. Plates were judged to be acceptable if at least 85% of the compounds on the plate were at least 85% full length.

#### Example 9

**[0162]** Cell Culture and Oligonucleotide Treatment

**[0163]** The effect of antisense compounds on target nucleic acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. This can be routinely determined using,

for example, PCR or Northern blot analysis. The following cell types are provided for illustrative purposes, but other cell types can be routinely used, provided that the target is expressed in the cell type chosen. This can be readily determined by methods routine in the art, for example Northern blot analysis, ribonuclease protection assays, or RT-PCR.

**[0164]** T-24 Cells:

**[0165]** The human transitional cell bladder carcinoma cell line T-24 was obtained from the American Type Culture Collection (ATCC) (Manassas, Va.). T-24 cells were routinely cultured in complete McCoy's 5A basal media (Invitrogen Corporation, Carlsbad, Calif.) supplemented with 10% fetal calf serum (Invitrogen Corporation, Carlsbad, Calif.), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Invitrogen Corporation, Carlsbad, Calif.). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #353872) at a density of 7000 cells/well for use in RT-PCR analysis.

**[0166]** For Northern blotting or other analysis, cells may be seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.

**[0167]** A549 Cells:

**[0168]** The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (ATCC) (Manassas, Va.). A549 cells were routinely cultured in DMEM basal media (Invitrogen Corporation, Carlsbad, Calif.) supplemented with 10% fetal calf serum (Invitrogen Corporation, Carlsbad, Calif.), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Invitrogen Corporation, Carlsbad, Calif.). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence.

**[0169]** NHDF Cells:

**[0170]** Human neonatal dermal fibroblast (NHDF) were obtained from the Clonetics Corporation (Walkersville, Md.). NHDFs were routinely maintained in Fibroblast Growth Medium (Clonetics Corporation, Walkersville, Md.) supplemented as recommended by the supplier. Cells were maintained for up to 10 passages as recommended by the supplier.

**[0171]** HEK Cells:

**[0172]** Human embryonic keratinocytes (HEK) were obtained from the Clonetics Corporation (Walkersville, Md.). HEKs were routinely maintained in Keratinocyte Growth Medium (Clonetics Corporation, Walkersville, Md.) formulated as recommended by the supplier. Cells were routinely maintained for up to 10 passages as recommended by the supplier.

**[0173]** Treatment with Antisense Compounds:

**[0174]** When cells reached 65-75% confluency, they were treated with oligonucleotide. For cells grown in 96-well plates, wells were washed once with 100  $\mu$ L OPTI-MEM™-1 reduced-serum medium (Invitrogen Corporation, Carlsbad, Calif.) and then treated with 130  $\mu$ L of OPTI-MEM™-1 containing 3.75  $\mu$ g/mL LIPOFECTIN™ (Invitrogen Corporation, Carlsbad, Calif.) and the desired con-

centration of oligonucleotide. Cells are treated and data are obtained in triplicate. After 4-7 hours of treatment at 37° C., the medium was replaced with fresh medium. Cells were harvested 16-24 hours after oligonucleotide treatment.

**[0175]** The concentration of oligonucleotide used varies from cell line to cell line. To determine the optimal oligonucleotide concentration for a particular cell line, the cells are treated with a positive control oligonucleotide at a range of concentrations. For human cells the positive control oligonucleotide is selected from either ISIS 13920 (TCCGT-CATCGCTCCTCAGGG, SEQ ID NO: 1) which is targeted to human H-ras, or ISIS 18078, (GTGCGCGCAGC-CCGAAATC, SEQ ID NO: 2) which is targeted to human Jun-N-terminal kinase-2 (JNK2). Both controls are 2'-O-methoxyethyl gapmers (2'-O-methoxyethyls shown in bold) with a phosphorothioate backbone. For mouse or rat cells the positive control oligonucleotide is ISIS 15770, ATGCAT-TCTGCCCCCAAGGA, SEQ ID NO: 3, a 2'-O-methoxyethyl gapmer (2'-O-methoxyethyls shown in bold) with a phosphorothioate backbone which is targeted to both mouse and rat c-ras. The concentration of positive control oligonucleotide that results in 80% inhibition of c-H-ras (for ISIS 13920), JNK2 (for ISIS 18078) or c-ras (for ISIS 15770) mRNA is then utilized as the screening concentration for new oligonucleotides in subsequent experiments for that cell line. If 80% inhibition is not achieved, the lowest concentration of positive control oligonucleotide that results in 60% inhibition of c-H-ras, JNK2 or c-ras mRNA is then utilized as the oligonucleotide screening concentration in subsequent experiments for that cell line. If 60% inhibition is not achieved, that particular cell line is deemed as unsuitable for oligonucleotide transfection experiments. The concentrations of antisense oligonucleotides used herein are from 50 nM to 300 nM.

#### Example 10

**[0176]** Analysis of Oligonucleotide Inhibition of STAT 6 Expression

**[0177]** Antisense modulation of STAT 6 expression can be assayed in a variety of ways known in the art. For example, STAT 6 mRNA levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time PCR (RT-PCR). Real-time quantitative PCR is presently preferred. RNA analysis can be performed on total cellular RNA or poly(A)<sup>+</sup> mRNA. The preferred method of RNA analysis of the present invention is the use of total cellular RNA as described in other examples herein. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available ABI PRISM™ 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, Calif. and used according to manufacturer's instructions.

**[0178]** Protein levels of STAT 6 can be quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA) or fluorescence-activated cell sorting (FACS). Antibodies directed to STAT 6 can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, Mich.), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art.

#### Example 11

**[0179]** Design of Phenotypic Assays and in vivo Studies for the use of STAT 6 Inhibitors

**[0180]** Phenotypic Assays

**[0181]** Once STAT 6 inhibitors have been identified by the methods disclosed herein, the compounds are further investigated in one or more phenotypic assays, each having measurable endpoints predictive of efficacy in the treatment of a particular disease state or condition.

**[0182]** Phenotypic assays, kits and reagents for their use are well known to those skilled in the art and are herein used to investigate the role and/or association of STAT 6 in health and disease. Representative phenotypic assays, which can be purchased from any one of several commercial vendors, include those for determining cell viability, cytotoxicity, proliferation or cell survival (Molecular Probes, Eugene, Oreg.; PerkinElmer, Boston, Mass.), protein-based assays including enzymatic assays (Panvera, LLC, Madison, Wis.; BD Biosciences, Franklin Lakes, N.J.; Oncogene Research Products, San Diego, Calif.), cell regulation, signal transduction, inflammation, oxidative processes and apoptosis (Assay Designs Inc., Ann Arbor, Mich.), triglyceride accumulation (Sigma-Aldrich, St. Louis, Mo.), angiogenesis assays, tube formation assays, cytokine and hormone assays and metabolic assays (Chemicon International Inc., Temecula, Calif.; Amersham Biosciences, Piscataway, N.J.).

**[0183]** In one non-limiting example, cells determined to be appropriate for a particular phenotypic assay (i.e., MCF-7 cells selected for breast cancer studies; adipocytes for obesity studies) are treated with STAT 6 inhibitors identified from the in vitro studies as well as control compounds at optimal concentrations which are determined by the methods described above. At the end of the treatment period, treated and untreated cells are analyzed by one or more methods specific for the assay to determine phenotypic outcomes and endpoints.

**[0184]** Phenotypic endpoints include changes in cell morphology over time or treatment dose as well as changes in levels of cellular components such as proteins, lipids, nucleic acids, hormones, saccharides or metals. Measurements of cellular status which include pH, stage of the cell cycle, intake or excretion of biological indicators by the cell, are also endpoints of interest.

**[0185]** Analysis of the genotype of the cell (measurement of the expression of one or more of the genes of the cell) after treatment is also used as an indicator of the efficacy or potency of the STAT 6 inhibitors. Hallmark genes, or those genes suspected to be associated with a specific disease state, condition, or phenotype, are measured in both treated and untreated cells.

**[0186]** In vivo Studies

**[0187]** The individual subjects of the in vivo studies described herein are warm-blooded vertebrate animals, which includes humans.

**[0188]** The clinical trial is subjected to rigorous controls to ensure that individuals are not unnecessarily put at risk and that they are fully informed about their role in the study. To account for the psychological effects of receiving treatments, volunteers are randomly given placebo or STAT 6

inhibitor. Furthermore, to prevent the doctors from being biased in treatments, they are not informed as to whether the medication they are administering is a STAT 6 inhibitor or a placebo. Using this randomization approach, each volunteer has the same chance of being given either the new treatment or the placebo.

**[0189]** Volunteers receive either the STAT 6 inhibitor or placebo for eight week period with biological parameters associated with the indicated disease state or condition being measured at the beginning (baseline measurements before any treatment), end (after the final treatment), and at regular intervals during the study period. Such measurements include the levels of nucleic acid molecules encoding STAT 6 or STAT 6 protein levels in body fluids, tissues or organs compared to pre-treatment levels. Other measurements include, but are not limited to, indices of the disease state or condition being treated, body weight, blood pressure, serum titers of pharmacologic indicators of disease or toxicity as well as ADME (absorption, distribution, metabolism and excretion) measurements.

**[0190]** Information recorded for each patient includes age (years), gender, height (cm), family history of disease state or condition (yes/no), motivation rating (some/moderate/great) and number and type of previous treatment regimens for the indicated disease or condition.

**[0191]** Volunteers taking part in this study are healthy adults (age 18 to 65 years) and roughly an equal number of males and females participate in the study. Volunteers with certain characteristics are equally distributed for placebo and STAT 6 inhibitor treatment. In general, the volunteers treated with placebo have little or no response to treatment, whereas the volunteers treated with the STAT 6 inhibitor show positive trends in their disease state or condition index at the conclusion of the study.

#### Example 12

**[0192]** RNA Isolation

**[0193]** Poly(A)+ mRNA Isolation

**[0194]** Poly(A)+ mRNA was isolated according to Miura et al., (*Clin. Chem.*, 1996, 42, 1758-1764). Other methods for poly(A)+ mRNA isolation are routine in the art. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200  $\mu$ L cold PBS. 60  $\mu$ L lysis buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA, 0.5 M NaCl, 0.5% NP-40, 20 mM vanadyl-ribonucleoside complex) was added to each well, the plate was gently agitated and then incubated at room temperature for five minutes. 55  $\mu$ L of lysate was transferred to Oligo d(T) coated 96-well plates (AGCT Inc., Irvine Calif.). Plates were incubated for 60 minutes at room temperature, washed 3 times with 200  $\mu$ L of wash buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.3 M NaCl). After the final wash, the plate was blotted on paper towels to remove excess wash buffer and then air-dried for 5 minutes. 60  $\mu$ L of elution buffer (5 mM Tris-HCl pH 7.6), preheated to 70° C., was added to each well, the plate was incubated on a 90° C. hot plate for 5 minutes, and the eluate was then transferred to a fresh 96-well plate.

**[0195]** Cells grown on 100 mm or other standard plates may be treated similarly, using appropriate volumes of all solutions.

**[0196]** Total RNA Isolation

**[0197]** Total RNA was isolated using an RNEASY 96™ kit and buffers purchased from Qiagen Inc. (Valencia, Calif.) following the manufacturer's recommended procedures. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200  $\mu$ L cold PBS. 150  $\mu$ L Buffer RLT was added to each well and the plate vigorously agitated for 20 seconds. 150  $\mu$ L of 70% ethanol was then added to each well and the contents mixed by pipetting three times up and down. The samples were then transferred to the RNEASY 96™ well plate attached to a QIAVAC™ manifold fitted with a waste collection tray and attached to a vacuum source. Vacuum was applied for 1 minute. 500  $\mu$ L of Buffer RW1 was added to each well of the RNEASY 96™ plate and incubated for 15 minutes and the vacuum was again applied for 1 minute. An additional 500  $\mu$ L of Buffer RW1 was added to each well of the RNEASY 96™ plate and the vacuum was applied for 2 minutes. 1 mL of Buffer RPE was then added to each well of the RNEASY 96™ plate and the vacuum applied for a period of 90 seconds. The Buffer RPE wash was then repeated and the vacuum was applied for an additional 3 minutes. The plate was then removed from the QIAVAC™ manifold and blotted dry on paper towels. The plate was then re-attached to the QIAVAC™ manifold fitted with a collection tube rack containing 1.2 mL collection tubes. RNA was then eluted by pipetting 140  $\mu$ L of RNase free water into each well, incubating 1 minute, and then applying the vacuum for 3 minutes.

**[0198]** The repetitive pipetting and elution steps may be automated using a QIAGEN Bio-Robot 9604 (Qiagen, Inc., Valencia Calif.). Essentially, after lysing of the cells on the culture plate, the plate is transferred to the robot deck where the pipetting, DNase treatment and elution steps are carried out.

#### Example 13

**[0199]** Real-time Quantitative PCR Analysis of STAT 6 mRNA Levels

**[0200]** Quantitation of STAT 6 mRNA levels was accomplished by real-time quantitative PCR using the ABI PRISM™ 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, Calif.) according to manufacturer's instructions. This is a closed-tube, non-gel-based, fluorescence detection system which allows high-throughput quantitation of polymerase chain reaction (PCR) products in real-time. As opposed to standard PCR in which amplification products are quantitated after the PCR is completed, products in real-time quantitative PCR are quantitated as they accumulate. This is accomplished by including in the PCR reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent dyes. A reporter dye (e.g., FAM or JOE, obtained from either PE-Applied Biosystems, Foster City, Calif., Operon Technologies Inc., Alameda, Calif. or Integrated DNA Technologies Inc., Coralville, Iowa) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either PE-Applied Biosystems, Foster City, Calif., Operon Technologies Inc., Alameda, Calif. or Integrated DNA Technologies Inc., Coralville, Iowa) is attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission

is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a substrate that can be cleaved by the 5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a sequence-specific fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the fluorescence intensity is monitored at regular intervals by laser optics built into the ABI PRISM™ Sequence Detection System. In each assay, a series of parallel reactions containing serial dilutions of mRNA from untreated control samples generates a standard curve that is used to quantitate the percent inhibition after antisense oligonucleotide treatment of test samples.

[0201] Prior to quantitative PCR analysis, primer-probe sets specific to the target gene being measured are evaluated for their ability to be "multiplexed" with a GAPDH amplification reaction. In multiplexing, both the target gene and the internal standard gene GAPDH are amplified concurrently in a single sample. In this analysis, mRNA isolated from untreated cells is serially diluted. Each dilution is amplified in the presence of primer-probe sets specific for GAPDH only, target gene only ("single-plexing"), or both (multiplexing). Following PCR amplification, standard curves of GAPDH and target mRNA signal as a function of dilution are generated from both the single-plexed and multiplexed samples. If both the slope and correlation coefficient of the GAPDH and target signals generated from the multiplexed samples fall within 10% of their corresponding values generated from the single-plexed samples, the primer-probe set specific for that target is deemed multiplexable. Other methods of PCR are also known in the art.

[0202] PCR reagents were obtained from Invitrogen Corporation, (Carlsbad, Calif.). RT-PCR reactions were carried out by adding 20  $\mu$ L PCR cocktail (2.5 $\times$ PCR buffer minus  $MgCl_2$ , 6.6 mM  $MgCl_2$ , 375  $\mu$ M each of dATP, dCTP, dCTP and dGTP, 375 nM each of forward primer and reverse primer, 125 nM of probe, 4 Units RNase inhibitor, 1.25 Units PLATINUM® Taq, 5 Units MuLV reverse transcriptase, and 2.5 $\times$  ROX dye) to 96-well plates containing 30  $\mu$ L total RNA solution (20-200 ng). The RT reaction was carried out by incubation for 30 minutes at 48° C. Following a 10 minute incubation at 95° C. to activate the PLATINUM® Taq, 40 cycles of a two-step PCR protocol were carried out: 95° C. for 15 seconds (denaturation) followed by 60° C. for 1.5 minutes (annealing/extension).

[0203] Gene target quantities obtained by real time RT-PCR are normalized using either the expression level of GAPDH, a gene whose expression is constant, or by quantifying total RNA using RiboGreen™ (Molecular Probes, Inc. Eugene, Oreg.). GAPDH expression is quantified by real time RT-PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, Oreg.). Methods of RNA quantification by RiboGreen™ are taught in Jones, L. J., et al, (Analytical Biochemistry, 1998, 265, 368-374).

[0204] In this assay, 170  $\mu$ L of RiboGreen™ working reagent (RiboGreen™ reagent diluted 1:350 in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5) is pipetted into a 96-well plate containing 30  $\mu$ L purified, cellular RNA. The plate is read in a CytoFluor 4000 (PE Applied Biosystems) with excitation at 485 nm and emission at 530 nm.

[0205] Probes and primers to human STAT 6 were designed to hybridize to a human STAT 6 sequence, using published sequence information (GenBank accession number NM\_003153.1, incorporated herein as SEQ ID NO:4). For human STAT 6 the PCR primers were:

[0206] forward primer: CCAAACGCTGTCTCCGGA (SEQ ID NO: 5)

[0207] reverse primer: GCTAGTAACGTACTGTTTGCTGATGAA (SEQ ID NO: 6) and the PCR probe was: FAM-CTACTGGTCTGACCGGCTGATCATTTGG-TAMRA (SEQ ID NO: 7) where FAM is the fluorescent dye and TAMRA is the quencher dye. For human GAPDH the PCR primers were:

[0208] forward primer: GAAGGTGAAGGTCGAGATC (SEQ ID NO:8)

[0209] reverse primer: GAAGATGGTGATGGATTTC (SEQ ID NO:9) and the PCR probe was: 5' JOE-CAAGCTTCCCCTTCTCAGCC-TAMRA 3' (SEQ ID NO: 10) where JOE is the fluorescent reporter dye and TAMRA is the quencher dye.

#### Example 14

[0210] Northern Blot Analysis of STAT 6 mRNA Levels

[0211] Eighteen hours after antisense treatment, cell monolayers were washed twice with cold PBS and lysed in 1 mL RNAzol™ (TEL-TEST "B" Inc., Friendswood, Tex.). Total RNA was prepared following manufacturer's recommended protocols. Twenty micrograms of total RNA was fractionated by electrophoresis through 1.2% agarose gels containing 1.1% formaldehyde using a MOPS buffer system (AMRESCO, Inc. Solon, Ohio). RNA was transferred from the gel to HYBOND™-N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway, N.J.) by overnight capillary transfer using a Northern/Southern Transfer buffer system (TEL-TEST "B" Inc., Friendswood, Tex.). RNA transfer was confirmed by UV visualization. Membranes were fixed by UV cross-linking using a STRATALINKER™ UV Crosslinker 2400 (Stratagene, Inc., La Jolla, Calif.) and then probed using QUICKHYB™ hybridization solution (Stratagene, La Jolla, Calif.) using manufacturer's recommendations for stringent conditions.

[0212] To detect human STAT 6, a human STAT 6 specific probe was prepared by PCR using the forward primer CCAAACGCTGTCTCCGGA (SEQ ID NO: 5) and the reverse primer GCTAGTAACGTACTGTTTGCTGATGAA (SEQ ID NO: 6). To normalize for variations in loading and transfer efficiency membranes were stripped and probed for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto, Calif.).

[0213] Hybridized membranes were visualized and quantitated using a PHOSPHORIMAGER™ and IMAGEQUANT™ Software V3.3 (Molecular Dynamics, Sunnyvale, Calif.). Data was normalized to GAPDH levels in untreated controls.

#### Example 15

[0214] Antisense Inhibition of Human STAT 6 Expression by Chimeric Phosphorothioate Oligonucleotides having 2'-MOE Wings and a Deoxy Gap

[0215] In accordance with the present invention, a series of antisense compounds were designed to target different

regions of the human STAT 6 RNA, using published sequences (GenBank accession number NM\_003153.1, incorporated herein as SEQ ID NO: 4; GenBank accession number BC005823.1, incorporated herein as SEQ ID NO: 11; a genomic sequence of human STAT 6 represented by the complement of residues 157501-174000 of GenBank accession number AC018673.4, incorporated herein as SEQ ID NO: 12; GenBank accession number BE972840.1, the complement of which is incorporated herein as SEQ ID NO: 13; and GenBank accession number BF902909.1, the complement of which is incorporated herein as SEQ ID NO: 14). The compounds are shown in Table 1. "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the compound binds. All compounds in Table 1 are chimeric oligonucleotides ("gap-

mers") 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds were analyzed for their effect on human STAT 6 mRNA levels by quantitative real-time PCR as described in other examples herein. Data are averages from three experiments in which T-24 cells were treated with the oligonucleotides of the present invention. The positive control for each datapoint is identified in the table by sequence ID number. If present, "N.D." indicates "no data".

TABLE 1

| Inhibition of human STAT 6 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap |        |           |             |                       |         |           |           |  |
|--------------------------------------------------------------------------------------------------------------------------|--------|-----------|-------------|-----------------------|---------|-----------|-----------|--|
| ISIS #                                                                                                                   | REGION | TARGET    | TARGET SITE | SEQUENCE              | % INHIB | CONTROL   |           |  |
|                                                                                                                          |        | SEQ ID NO |             |                       |         | SEQ ID NO | SEQ ID NO |  |
| 153765                                                                                                                   | Coding | 4         | 1951        | agtgagcgaatggacaggtc  | 96      | 15        | 2         |  |
| 153766                                                                                                                   | Coding | 4         | 2483        | cgctgtcactggctggctca  | 83      | 16        | 2         |  |
| 153767                                                                                                                   | Coding | 4         | 1171        | ttgatgatttctccagtgtc  | 92      | 17        | 2         |  |
| 153768                                                                                                                   | Coding | 4         | 940         | aggacttcattccagccggcc | 50      | 18        | 2         |  |
| 153769                                                                                                                   | Coding | 4         | 1054        | cccaggaacctcaagcccaa  | 68      | 19        | 2         |  |
| 153770                                                                                                                   | Coding | 4         | 242         | gtcaccagaagatgccgca   | 53      | 20        | 2         |  |
| 153771                                                                                                                   | Coding | 4         | 2100        | tttccacggctcatcttgatg | 59      | 21        | 2         |  |
| 153772                                                                                                                   | Coding | 4         | 379         | aagatggtgctcccctcccc  | 34      | 22        | 2         |  |
| 153773                                                                                                                   | Coding | 4         | 765         | gccgtttccaaatctggatc  | 76      | 23        | 2         |  |
| 153774                                                                                                                   | Coding | 4         | 729         | ctttgggtgcctctagctct  | 89      | 24        | 2         |  |
| 153775                                                                                                                   | Coding | 4         | 1725        | gtttggtgaggtccaggaca  | 89      | 25        | 2         |  |
| 153776                                                                                                                   | Coding | 4         | 2321        | catctgcaggtaggctcct   | 85      | 26        | 2         |  |
| 153777                                                                                                                   | Coding | 4         | 2676        | tggcccttaggtccatgtgg  | 76      | 27        | 2         |  |
| 153778                                                                                                                   | Coding | 4         | 1905        | ctatctgtggagagcatcc   | 72      | 28        | 2         |  |
| 153779                                                                                                                   | Coding | 4         | 1805        | attgagaagaaggctagtaa  | 83      | 29        | 2         |  |
| 153780                                                                                                                   | Coding | 4         | 392         | gctgatgtgtgcaagatgg   | 78      | 30        | 2         |  |
| 153781                                                                                                                   | 3'UTR  | 4         | 3019        | gccccatcacctcagagag   | 80      | 31        | 2         |  |
| 153782                                                                                                                   | Coding | 4         | 417         | ccctctgatatatgtctca   | 73      | 32        | 2         |  |
| 153783                                                                                                                   | Coding | 4         | 1797        | gaaggctagtaacgtactgt  | 84      | 33        | 2         |  |
| 153784                                                                                                                   | Coding | 4         | 1819        | gttccgtcgggctcattgag  | 92      | 34        | 2         |  |
| 153785                                                                                                                   | Coding | 4         | 2479        | gtcactggctggctcaggca  | 87      | 35        | 2         |  |
| 153786                                                                                                                   | Coding | 4         | 1513        | ttcagagtttcacacatctt  | 83      | 36        | 2         |  |
| 153787                                                                                                                   | 5'UTR  | 4         | 79          | caggccccataggtctgtag  | 88      | 37        | 2         |  |
| 153788                                                                                                                   | Coding | 4         | 644         | tatcaagctgtgcagagaca  | 80      | 38        | 2         |  |
| 153789                                                                                                                   | Coding | 4         | 272         | caggaaactcccagggtggc  | 74      | 39        | 2         |  |

TABLE 1-continued

| Inhibition of human STAT 6 mRNA levels by chimeric<br>phosphorothioate oligonucleotides having 2'-MOE wings and a<br>deoxy gap |                |           |        |                        |         |        |           |
|--------------------------------------------------------------------------------------------------------------------------------|----------------|-----------|--------|------------------------|---------|--------|-----------|
| ISIS #                                                                                                                         | REGION         | TARGET    | TARGET | SEQUENCE               | % INHIB | SEQ ID | CONTROL   |
|                                                                                                                                |                | SEQ ID NO | SITE   |                        |         | NO     | SEQ ID NO |
| 153790                                                                                                                         | 3'UTR          | 4         | 3000   | gctctgtatgtgtgtgtgcg   | 90      | 40     | 2         |
| 153791                                                                                                                         | Coding         | 4         | 1972   | agatcccggttcgggtcccc   | 88      | 41     | 2         |
| 153792                                                                                                                         | Coding         | 4         | 794    | cgggtgcgcattccctgccca  | 94      | 42     | 2         |
| 153793                                                                                                                         | Coding         | 4         | 1997   | gggatatagatTTTTGagct   | 53      | 43     | 2         |
| 153794                                                                                                                         | Start<br>Codon | 4         | 146    | gatctgggacttgagggtttg  | 71      | 44     | 2         |
| 153795                                                                                                                         | Coding         | 4         | 2165   | tccaaggtcataagaaggca   | 88      | 45     | 2         |
| 153796                                                                                                                         | Coding         | 4         | 1762   | atgatcagccgggtcagacca  | 84      | 46     | 2         |
| 153797                                                                                                                         | Coding         | 4         | 1205   | cccaggaatgctgtttctcca  | 88      | 47     | 2         |
| 153798                                                                                                                         | Coding         | 4         | 944    | tctcaggacttcatccagcc   | 6       | 48     | 2         |
| 153799                                                                                                                         | Coding         | 4         | 1671   | ccagcaggatctccttggtg   | 82      | 49     | 2         |
| 153800                                                                                                                         | Coding         | 4         | 1160   | tccagtgcctttctgctccag  | 87      | 50     | 2         |
| 153801                                                                                                                         | Coding         | 4         | 328    | acagtgtctgaaagtagggc   | 50      | 51     | 2         |
| 195427                                                                                                                         | 5'UTR          | 4         | 39     | gctggccctgctagcacctc   | 68      | 52     | 2         |
| 195428                                                                                                                         | Start<br>Codon | 4         | 158    | ccacagagacatgatctggg   | 79      | 53     | 2         |
| 195429                                                                                                                         | Coding         | 4         | 541    | gtcttaaacttgagttcttc   | 53      | 54     | 2         |
| 195430                                                                                                                         | Coding         | 4         | 718    | tctagctctccagtggctctc  | 78      | 55     | 2         |
| 195431                                                                                                                         | Coding         | 4         | 1085   | ggccctgaccagcggaggct   | 72      | 56     | 2         |
| 195432                                                                                                                         | Coding         | 4         | 1290   | cctctgtgacagactcagtg   | 72      | 57     | 2         |
| 195433                                                                                                                         | Coding         | 4         | 615    | tccatactgaggctgttgctc  | 20      | 58     | 2         |
| 195434                                                                                                                         | Coding         | 4         | 1887   | cctggccccggatgacatgg   | 53      | 59     | 2         |
| 195435                                                                                                                         | Coding         | 4         | 2152   | gaaggcaccatggttagcoat  | 55      | 60     | 2         |
| 195436                                                                                                                         | Coding         | 4         | 2506   | ccaatccaagtgcctgagg    | 70      | 61     | 2         |
| 195437                                                                                                                         | Stop<br>Codon  | 4         | 2699   | cagctgggatcaccaactgg   | 49      | 62     | 2         |
| 195438                                                                                                                         | 3'UTR          | 4         | 2944   | gtgtctcagagcctgaactt   | 77      | 63     | 2         |
| 195439                                                                                                                         | 5'UTR          | 11        | 23     | taagcagtggctgcccagc    | 51      | 64     | 2         |
| 195440                                                                                                                         | 5'UTR          | 11        | 38     | cctccctcttcagtgtgaagc  | 65      | 65     | 2         |
| 195441                                                                                                                         | 3'UTR          | 11        | 3185   | agaagccttccatgccctaa   | 83      | 66     | 2         |
| 195442                                                                                                                         | 3'UTR          | 11        | 3230   | tatgttcctgcctatccgtc   | 76      | 67     | 2         |
| 195443                                                                                                                         | 3'UTR          | 11        | 3531   | caactaagggtgccagctata  | 86      | 68     | 2         |
| 195444                                                                                                                         | 3'UTR          | 11        | 3539   | tggatcatgcaactaagggtgc | 84      | 69     | 2         |
| 195445                                                                                                                         | 3'UTR          | 11        | 3585   | atttgtgtgtgcacgtaggc   | 84      | 70     | 2         |
| 195446                                                                                                                         | 3'UTR          | 11        | 3599   | tctcaccctcccaaatttgt   | 48      | 71     | 2         |

TABLE 1-continued

| Inhibition of human STAT 6 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap |                             |           |        |                      |         |           |           |
|--------------------------------------------------------------------------------------------------------------------------|-----------------------------|-----------|--------|----------------------|---------|-----------|-----------|
| ISIS #                                                                                                                   | REGION                      | TARGET    | TARGET | SEQUENCE             | % INHIB | CONTROL   |           |
|                                                                                                                          |                             | SEQ ID NO | SITE   |                      |         | SEQ ID NO | SEQ ID NO |
| 195447                                                                                                                   | 3'UTR                       | 11        | 3629   | agcacacttgctgctgtctt | 74      | 72        | 2         |
| 195448                                                                                                                   | 3'UTR                       | 11        | 3779   | gccaggcctggaccagact  | 60      | 73        | 2         |
| 195449                                                                                                                   | 3'UTR                       | 11        | 3835   | gggcaacagaaaagatgcag | 50      | 74        | 2         |
| 195450                                                                                                                   | Intron                      | 12        | 2812   | aatgtcagcttttaatctgt | 67      | 75        | 2         |
| 195451                                                                                                                   | Intron                      | 12        | 3082   | gagtcaatgcctgagatggg | 50      | 76        | 2         |
| 195452                                                                                                                   | Intron:<br>Exon<br>Junction | 12        | 6200   | caggaagcaactgggagtga | 7       | 77        | 2         |
| 195453                                                                                                                   | Exon:<br>Intron<br>Junction | 12        | 8677   | ccatctcagagaaggcattg | 81      | 78        | 2         |
| 195454                                                                                                                   | Intron                      | 12        | 10476  | tgcacatgtccctgtgggat | 64      | 79        | 2         |
| 195455                                                                                                                   | Exon:<br>Intron<br>Junction | 12        | 11486  | gggactcaccggtcagacca | 26      | 80        | 2         |
| 195456                                                                                                                   | Intron:<br>Exon<br>Junction | 12        | 12582  | agtggttggtccctggagga | 71      | 81        | 2         |
| 195457                                                                                                                   | Exon:<br>Intron<br>Junction | 12        | 12691  | agtccttacaccatatctg  | 0       | 82        | 2         |
| 195458                                                                                                                   | Genomic                     | 13        | 9      | caaagtgtggaagtgaagg  | 0       | 83        | 2         |
| 195459                                                                                                                   | Genomic                     | 13        | 148    | ctctggtggccacggtgga  | 0       | 84        | 2         |
| 195460                                                                                                                   | Genomic                     | 13        | 654    | ggtgtatggctgctcagact | 67      | 85        | 2         |
| 195461                                                                                                                   | Genomic                     | 14        | 66     | aggaggtacatgtgactgac | 23      | 86        | 2         |

[0216] As shown in Table 1, SEQ ID NOs: 15, 16, 17, 19, 23, 24, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 42, 44, 45, 46, 47, 49, 50, 52, 53, 55, 56, 57, 61, 66, 67, 68, 69, 70, 72, 73, 75, 78, 79, 81 and 85 rated at least 60% inhibition of human STAT 6 expression in this assay and are therefore preferred. More preferred are SEQ ID NOs: 15, 34 and 42. The target regions to which these preferred sequences are complementary are herein referred to as “preferred target segments” and are therefore preferred for targeting by com-

pounds of the present invention. These preferred target segments are shown in Table 2. The sequences represent the reverse complement of the preferred antisense compounds shown in Table 1. “Target site” indicates the first (5'-most) nucleotide number on the particular target nucleic acid to which the oligonucleotide binds. Also shown in Table 2 is the species in which each of the preferred target segments was found.

TABLE 2

| Sequence and position of preferred target segments identified in STAT 6. |           |        |                      |          |                   |           |  |
|--------------------------------------------------------------------------|-----------|--------|----------------------|----------|-------------------|-----------|--|
| SITEID                                                                   | TARGET    | TARGET | SEQUENCE             | REV COMP |                   | SEQ ID NO |  |
|                                                                          | SEQ ID NO | SITE   |                      | OF SEQ   | ID ACTIVE IN      |           |  |
| 69306                                                                    | 4         | 1951   | gacctgtccattcgctcact | 15       | <i>H. sapiens</i> | 87        |  |
| 69307                                                                    | 4         | 2483   | tgagccagccagtgacagcg | 16       | <i>H. sapiens</i> | 88        |  |

TABLE 2-continued

| Sequence and position of preferred target segments identified in STAT 6. |                        |                |                       |                       |                   |              |
|--------------------------------------------------------------------------|------------------------|----------------|-----------------------|-----------------------|-------------------|--------------|
| SITEID                                                                   | TARGET<br>SEQ ID<br>NO | TARGET<br>SITE | SEQUENCE              | REV COMP<br>OF SEQ ID | ACTIVE IN         | SEQ ID<br>NO |
| 69308                                                                    | 4                      | 1171           | agcactggagaaatcatcaa  | 17                    | <i>H. sapiens</i> | 89           |
| 69310                                                                    | 4                      | 1054           | ttgggcttgaggttcctggg  | 19                    | <i>H. sapiens</i> | 90           |
| 69314                                                                    | 4                      | 765            | gatccagatttggaacggc   | 23                    | <i>H. sapiens</i> | 91           |
| 69315                                                                    | 4                      | 729            | agagctagaggcagccaaag  | 24                    | <i>H. sapiens</i> | 92           |
| 69316                                                                    | 4                      | 1725           | tgtcctggacctcaccaaac  | 25                    | <i>H. sapiens</i> | 93           |
| 69317                                                                    | 4                      | 2321           | aggagcctcacctgcagatg  | 26                    | <i>H. sapiens</i> | 94           |
| 69318                                                                    | 4                      | 2676           | ccacatggacctaaaggcca  | 27                    | <i>H. sapiens</i> | 95           |
| 69319                                                                    | 4                      | 1905           | ggatggctctccacagatag  | 28                    | <i>H. sapiens</i> | 96           |
| 69320                                                                    | 4                      | 1805           | ttactagccttcttctcaat  | 29                    | <i>H. sapiens</i> | 97           |
| 69321                                                                    | 4                      | 392            | ccatcttgcaacacatcagc  | 30                    | <i>H. sapiens</i> | 98           |
| 69322                                                                    | 4                      | 3019           | ctctctgagggatgaggggc  | 31                    | <i>H. sapiens</i> | 99           |
| 69323                                                                    | 4                      | 417            | tgagagcatatatcagaggg  | 32                    | <i>H. sapiens</i> | 100          |
| 69324                                                                    | 4                      | 1797           | acagtacgttactagccttc  | 33                    | <i>H. sapiens</i> | 101          |
| 69325                                                                    | 4                      | 1819           | ctcaatgagcccgacggaac  | 34                    | <i>H. sapiens</i> | 102          |
| 69326                                                                    | 4                      | 2479           | tgcctgagccagccagtgc   | 35                    | <i>H. sapiens</i> | 103          |
| 69327                                                                    | 4                      | 1513           | aagatgtgtgaaactctgaa  | 36                    | <i>H. sapiens</i> | 104          |
| 69328                                                                    | 4                      | 79             | ctacagacctatggggcctg  | 37                    | <i>H. sapiens</i> | 105          |
| 69329                                                                    | 4                      | 644            | tgtctctgcacagcttgata  | 38                    | <i>H. sapiens</i> | 106          |
| 69330                                                                    | 4                      | 272            | gccagccctgggagttcctg  | 39                    | <i>H. sapiens</i> | 107          |
| 69331                                                                    | 4                      | 3000           | cgcacacacacatacagagc  | 40                    | <i>H. sapiens</i> | 108          |
| 69332                                                                    | 4                      | 1972           | ggggaccgaatccgggatct  | 41                    | <i>H. sapiens</i> | 109          |
| 69333                                                                    | 4                      | 794            | tggcaggggaatggcgaccg  | 42                    | <i>H. sapiens</i> | 110          |
| 69335                                                                    | 4                      | 146            | caacctccaagtcccagatc  | 44                    | <i>H. sapiens</i> | 111          |
| 69336                                                                    | 4                      | 2165           | tgccttcttatgaccttgga  | 45                    | <i>H. sapiens</i> | 112          |
| 69337                                                                    | 4                      | 1762           | tggctctgaccggctgatcat | 46                    | <i>H. sapiens</i> | 113          |
| 69338                                                                    | 4                      | 1205           | tggagaacagcattcctggg  | 47                    | <i>H. sapiens</i> | 114          |
| 69340                                                                    | 4                      | 1671           | caacaaggagatcctgctgg  | 49                    | <i>H. sapiens</i> | 115          |
| 69341                                                                    | 4                      | 1160           | ctggagcagaaagcactgga  | 50                    | <i>H. sapiens</i> | 116          |
| 113659                                                                   | 4                      | 39             | gaggtgctagcaggccagc   | 52                    | <i>H. sapiens</i> | 117          |
| 113660                                                                   | 4                      | 158            | cccagatcatgtctctgtgg  | 53                    | <i>H. sapiens</i> | 118          |
| 113662                                                                   | 4                      | 718            | gagaccactggagagctaga  | 55                    | <i>H. sapiens</i> | 119          |
| 113663                                                                   | 4                      | 1085           | agcctccgctggctcagggcc | 56                    | <i>H. sapiens</i> | 120          |
| 113664                                                                   | 4                      | 1290           | cactgagtctgtcacagagg  | 57                    | <i>H. sapiens</i> | 121          |
| 113668                                                                   | 4                      | 2506           | cctcagggcacttggttgg   | 61                    | <i>H. sapiens</i> | 122          |
| 113670                                                                   | 4                      | 2944           | aagttcaggctctgagacac  | 63                    | <i>H. sapiens</i> | 123          |

TABLE 2-continued

| Sequence and position of preferred target segments identified in STAT 6. |                     |                |                      |                       |                   |
|--------------------------------------------------------------------------|---------------------|----------------|----------------------|-----------------------|-------------------|
| SITEID                                                                   | TARGET<br>SEQ ID NO | TARGET<br>SITE | SEQUENCE             | REV COMP<br>OF SEQ ID | SEQ ID<br>NO      |
|                                                                          |                     |                |                      | ACTIVE IN             |                   |
| 113672                                                                   | 11                  | 38             | gcttacctgaagaggagg   | 65                    | <i>H. sapiens</i> |
| 113673                                                                   | 11                  | 3185           | ttaggcatggaagccttct  | 66                    | <i>H. sapiens</i> |
| 113674                                                                   | 11                  | 3230           | gacggataggcaggaacata | 67                    | <i>H. sapiens</i> |
| 113675                                                                   | 11                  | 3531           | tatagctggcaccttagttg | 68                    | <i>H. sapiens</i> |
| 113676                                                                   | 11                  | 3539           | gcaccttagttgcatgacca | 69                    | <i>H. sapiens</i> |
| 113677                                                                   | 11                  | 3585           | gcctacgtgacaacacaaat | 70                    | <i>H. sapiens</i> |
| 113679                                                                   | 11                  | 3629           | aagacagcagcaagtgtgct | 72                    | <i>H. sapiens</i> |
| 113680                                                                   | 11                  | 3779           | agtctgggtccaggcctggc | 73                    | <i>H. sapiens</i> |
| 113682                                                                   | 12                  | 2812           | acagattaaaagctgacatt | 75                    | <i>H. sapiens</i> |
| 113685                                                                   | 12                  | 8677           | caatgccttctctgagatgg | 78                    | <i>H. sapiens</i> |
| 113686                                                                   | 12                  | 10476          | atcccacagggacatgtgca | 79                    | <i>H. sapiens</i> |
| 113688                                                                   | 12                  | 12582          | tcctccagggaccaaccact | 81                    | <i>H. sapiens</i> |
| 113692                                                                   | 13                  | 654            | agtctgagcagccatacacc | 85                    | <i>H. sapiens</i> |

[0217] As these “preferred target segments” have been found by experimentation to be open to, and accessible for, hybridization with the antisense compounds of the present invention, one of skill in the art will recognize or be able to ascertain, using no more than routine experimentation, further embodiments of the invention that encompass other compounds that specifically hybridize to these preferred target segments and consequently inhibit the expression of STAT 6.

[0218] According to the present invention, antisense compounds include antisense oligomeric compounds, antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other short oligomeric compounds which hybridize to at least a portion of the target nucleic acid.

Example 16

[0219] Western Blot Analysis of STAT 6 Protein Levels

[0220] Western blot analysis (immunoblot analysis) is carried out using standard methods. Cells are harvested 16-20 h after oligonucleotide treatment, washed once with PBS, suspended in Laemmli buffer (100 ul/well), boiled for 5 minutes and loaded on a 16% SDS-PAGE gel. Gels are run for 1.5 hours at 150 V, and transferred to membrane for western blotting. Appropriate primary antibody directed to STAT 6 is used, with a radiolabeled or fluorescently labeled secondary antibody directed against the primary antibody species. Bands are visualized using a PHOSPHORIMAGER™ (Molecular Dynamics, Sunnyvale Calif.).

Example 17

[0221] Targeting of Individual Oligonucleotides to Specific Variants of STAT 6

[0222] It is advantageous to selectively inhibit the expression of one or more variants of STAT 6. Consequently, in one embodiment of the present invention are oligonucleotides that selectively target, hybridize to, and specifically inhibit one or more, but fewer than all of the variants of STAT 6. A summary of the target sites of the variants is shown in Table 3 and includes GenBank accession number BC005823.1, representing STAT 6 main mRNA (represented in Table 3 as STAT 6), incorporated herein as SEQ ID NO: 11; GenBank accession number BE972840.1, representing STAT 6d, incorporated herein as SEQ ID NO: 13; GenBank accession number BF902909.1, representing STAT 6e, incorporated herein as SEQ ID NO: 14; GenBank accession number AR204914.1, representing STAT 6b, incorporated herein as SEQ ID NO: 137; and GenBank accession number AR204915.1, representing STAT 6c, incorporated herein as SEQ ID NO: 138.

TABLE 3

| Targeting of individual oligonucleotides to specific variants of STAT 6 |                        |                |         |                       |
|-------------------------------------------------------------------------|------------------------|----------------|---------|-----------------------|
| ISIS #                                                                  | OLIGO<br>SEQ ID<br>NO. | TARGET<br>SITE | VARIANT | VARIANT SEQ<br>ID NO. |
| 153765                                                                  | 15                     | 2066           | STAT 6  | 11                    |
| 153765                                                                  | 15                     | 1932           | STAT 6c | 137                   |
| 153765                                                                  | 15                     | 1741           | STAT 6b | 138                   |

TABLE 3-continued

| Targeting of individual oligonucleotides<br>to specific variants of STAT 6 |                        |                |         |                       |
|----------------------------------------------------------------------------|------------------------|----------------|---------|-----------------------|
| ISIS #                                                                     | OLIGO<br>SEQ ID<br>NO. | TARGET<br>SITE | VARIANT | VARIANT SEQ<br>ID NO. |
| 153766                                                                     | 16                     | 2597           | STAT 6  | 11                    |
| 153766                                                                     | 16                     | 2464           | STAT 6c | 137                   |
| 153766                                                                     | 16                     | 2273           | STAT 6b | 138                   |
| 153767                                                                     | 17                     | 1286           | STAT 6  | 11                    |
| 153767                                                                     | 17                     | 1236           | STAT 6c | 137                   |
| 153767                                                                     | 17                     | 961            | STAT 6b | 138                   |
| 153768                                                                     | 18                     | 1055           | STAT 6  | 11                    |
| 153768                                                                     | 18                     | 1005           | STAT 6c | 137                   |
| 153768                                                                     | 18                     | 730            | STAT 6b | 138                   |
| 153769                                                                     | 19                     | 1169           | STAT 6  | 11                    |
| 153769                                                                     | 19                     | 1119           | STAT 6c | 137                   |
| 153769                                                                     | 19                     | 844            | STAT 6b | 138                   |
| 153770                                                                     | 20                     | 357            | STAT 6  | 11                    |
| 153770                                                                     | 20                     | 307            | STAT 6c | 137                   |
| 153770                                                                     | 20                     | 171            | STAT 6b | 138                   |
| 153771                                                                     | 21                     | 2215           | STAT 6  | 11                    |
| 153771                                                                     | 21                     | 2081           | STAT 6c | 137                   |
| 153771                                                                     | 21                     | 1890           | STAT 6b | 138                   |
| 153772                                                                     | 22                     | 494            | STAT 6  | 11                    |
| 153772                                                                     | 22                     | 444            | STAT 6c | 137                   |
| 153773                                                                     | 23                     | 880            | STAT 6  | 11                    |
| 153773                                                                     | 23                     | 830            | STAT 6c | 137                   |
| 153773                                                                     | 23                     | 555            | STAT 6b | 138                   |
| 153774                                                                     | 24                     | 844            | STAT 6  | 11                    |
| 153774                                                                     | 24                     | 794            | STAT 6c | 137                   |
| 153774                                                                     | 24                     | 519            | STAT 6b | 138                   |
| 153775                                                                     | 25                     | 1840           | STAT 6  | 11                    |
| 153775                                                                     | 25                     | 1790           | STAT 6c | 137                   |
| 153775                                                                     | 25                     | 1515           | STAT 6b | 138                   |
| 153776                                                                     | 26                     | 2435           | STAT 6  | 11                    |
| 153776                                                                     | 26                     | 298            | STAT 6e | 14                    |
| 153776                                                                     | 26                     | 2302           | STAT 6c | 137                   |
| 153776                                                                     | 26                     | 2111           | STAT 6b | 138                   |
| 153777                                                                     | 27                     | 2790           | STAT 6  | 11                    |
| 153777                                                                     | 27                     | 2657           | STAT 6c | 137                   |
| 153777                                                                     | 27                     | 2466           | STAT 6b | 138                   |
| 153778                                                                     | 28                     | 2020           | STAT 6  | 11                    |
| 153778                                                                     | 28                     | 1886           | STAT 6c | 137                   |
| 153778                                                                     | 28                     | 1695           | STAT 6b | 138                   |
| 153779                                                                     | 29                     | 1920           | STAT 6  | 11                    |
| 153779                                                                     | 29                     | 1595           | STAT 6b | 138                   |
| 153780                                                                     | 30                     | 507            | STAT 6  | 11                    |
| 153780                                                                     | 30                     | 457            | STAT 6c | 137                   |
| 153781                                                                     | 31                     | 3133           | STAT 6  | 11                    |
| 153781                                                                     | 31                     | 3000           | STAT 6c | 137                   |
| 153781                                                                     | 31                     | 2809           | STAT 6b | 138                   |
| 153782                                                                     | 32                     | 532            | STAT 6  | 11                    |
| 153782                                                                     | 32                     | 482            | STAT 6c | 137                   |
| 153783                                                                     | 33                     | 1912           | STAT 6  | 11                    |
| 153783                                                                     | 33                     | 1587           | STAT 6b | 138                   |
| 153784                                                                     | 34                     | 1934           | STAT 6  | 11                    |
| 153784                                                                     | 34                     | 1609           | STAT 6b | 138                   |
| 153785                                                                     | 35                     | 2593           | STAT 6  | 11                    |
| 153785                                                                     | 35                     | 2460           | STAT 6c | 137                   |
| 153785                                                                     | 35                     | 2269           | STAT 6b | 138                   |
| 153786                                                                     | 36                     | 1628           | STAT 6  | 11                    |
| 153786                                                                     | 36                     | 377            | STAT 6d | 13                    |
| 153786                                                                     | 36                     | 1578           | STAT 6c | 137                   |
| 153786                                                                     | 36                     | 1303           | STAT 6b | 138                   |
| 153787                                                                     | 37                     | 194            | STAT 6  | 11                    |
| 153787                                                                     | 37                     | 76             | STAT 6c | 137                   |
| 153787                                                                     | 37                     | 8              | STAT 6b | 138                   |
| 153788                                                                     | 38                     | 759            | STAT 6  | 11                    |
| 153788                                                                     | 38                     | 709            | STAT 6c | 137                   |
| 153788                                                                     | 38                     | 434            | STAT 6b | 138                   |
| 153789                                                                     | 39                     | 387            | STAT 6  | 11                    |
| 153789                                                                     | 39                     | 337            | STAT 6c | 137                   |
| 153790                                                                     | 40                     | 3114           | STAT 6  | 11                    |
| 153790                                                                     | 40                     | 2981           | STAT 6c | 137                   |

TABLE 3-continued

| Targeting of individual oligonucleotides<br>to specific variants of STAT 6 |                        |                |         |                       |
|----------------------------------------------------------------------------|------------------------|----------------|---------|-----------------------|
| ISIS #                                                                     | OLIGO<br>SEQ ID<br>NO. | TARGET<br>SITE | VARIANT | VARIANT SEQ<br>ID NO. |
| 153790                                                                     | 40                     | 2790           | STAT 6b | 138                   |
| 153791                                                                     | 41                     | 2087           | STAT 6  | 11                    |
| 153791                                                                     | 41                     | 1953           | STAT 6c | 137                   |
| 153791                                                                     | 41                     | 1762           | STAT 6b | 138                   |
| 153792                                                                     | 42                     | 909            | STAT 6  | 11                    |
| 153792                                                                     | 42                     | 859            | STAT 6c | 137                   |
| 153792                                                                     | 42                     | 584            | STAT 6b | 138                   |
| 153793                                                                     | 43                     | 2112           | STAT 6  | 11                    |
| 153793                                                                     | 43                     | 1978           | STAT 6c | 137                   |
| 153793                                                                     | 43                     | 1787           | STAT 6b | 138                   |
| 153794                                                                     | 44                     | 261            | STAT 6  | 11                    |
| 153794                                                                     | 44                     | 211            | STAT 6c | 137                   |
| 153794                                                                     | 44                     | 75             | STAT 6b | 138                   |
| 153795                                                                     | 45                     | 2280           | STAT 6  | 11                    |
| 153795                                                                     | 45                     | 142            | STAT 6e | 14                    |
| 153795                                                                     | 45                     | 2146           | STAT 6c | 137                   |
| 153795                                                                     | 45                     | 1955           | STAT 6b | 138                   |
| 153796                                                                     | 46                     | 1877           | STAT 6  | 11                    |
| 153796                                                                     | 46                     | 1552           | STAT 6b | 138                   |
| 153797                                                                     | 47                     | 1320           | STAT 6  | 11                    |
| 153797                                                                     | 47                     | 1270           | STAT 6c | 137                   |
| 153797                                                                     | 47                     | 995            | STAT 6b | 138                   |
| 153798                                                                     | 48                     | 1059           | STAT 6  | 11                    |
| 153798                                                                     | 48                     | 1009           | STAT 6c | 137                   |
| 153798                                                                     | 48                     | 734            | STAT 6b | 138                   |
| 153799                                                                     | 49                     | 1786           | STAT 6  | 11                    |
| 153799                                                                     | 49                     | 535            | STAT 6d | 13                    |
| 153799                                                                     | 49                     | 1736           | STAT 6c | 137                   |
| 153799                                                                     | 49                     | 1461           | STAT 6b | 138                   |
| 153800                                                                     | 50                     | 1275           | STAT 6  | 11                    |
| 153800                                                                     | 50                     | 1225           | STAT 6c | 137                   |
| 153800                                                                     | 50                     | 950            | STAT 6b | 138                   |
| 153801                                                                     | 51                     | 443            | STAT 6  | 11                    |
| 153801                                                                     | 51                     | 393            | STAT 6c | 137                   |
| 195427                                                                     | 52                     | 154            | STAT 6  | 11                    |
| 195427                                                                     | 52                     | 36             | STAT 6c | 137                   |
| 195428                                                                     | 53                     | 273            | STAT 6  | 11                    |
| 195428                                                                     | 53                     | 223            | STAT 6c | 137                   |
| 195428                                                                     | 53                     | 87             | STAT 6b | 138                   |
| 195429                                                                     | 54                     | 656            | STAT 6  | 11                    |
| 195429                                                                     | 54                     | 606            | STAT 6c | 137                   |
| 195429                                                                     | 54                     | 331            | STAT 6b | 138                   |
| 195430                                                                     | 55                     | 833            | STAT 6  | 11                    |
| 195430                                                                     | 55                     | 783            | STAT 6c | 137                   |
| 195430                                                                     | 55                     | 508            | STAT 6b | 138                   |
| 195431                                                                     | 56                     | 1200           | STAT 6  | 11                    |
| 195431                                                                     | 56                     | 1150           | STAT 6c | 137                   |
| 195431                                                                     | 56                     | 875            | STAT 6b | 138                   |
| 195432                                                                     | 57                     | 1405           | STAT 6  | 11                    |
| 195432                                                                     | 57                     | 1355           | STAT 6c | 137                   |
| 195432                                                                     | 57                     | 1080           | STAT 6b | 138                   |
| 195433                                                                     | 58                     | 1730           | STAT 6  | 11                    |
| 195433                                                                     | 58                     | 479            | STAT 6d | 13                    |
| 195433                                                                     | 58                     | 1680           | STAT 6c | 137                   |
| 195433                                                                     | 58                     | 1405           | STAT 6b | 138                   |
| 195434                                                                     | 59                     | 2002           | STAT 6  | 11                    |
| 195434                                                                     | 59                     | 1868           | STAT 6c | 137                   |
| 195434                                                                     | 59                     | 1677           | STAT 6b | 138                   |
| 195435                                                                     | 60                     | 2267           | STAT 6  | 11                    |
| 195435                                                                     | 60                     | 129            | STAT 6e | 14                    |
| 195435                                                                     | 60                     | 2133           | STAT 6c | 137                   |
| 195435                                                                     | 60                     | 1942           | STAT 6b | 138                   |
| 195436                                                                     | 61                     | 2620           | STAT 6  | 11                    |
| 195436                                                                     | 61                     | 2487           | STAT 6c | 137                   |
| 195436                                                                     | 61                     | 2296           | STAT 6b | 138                   |
| 195437                                                                     | 62                     | 2813           | STAT 6  | 11                    |
| 195437                                                                     | 62                     | 2680           | STAT 6c | 137                   |
| 195437                                                                     | 62                     | 2489           | STAT 6b | 138                   |
| 195438                                                                     | 63                     | 3058           | STAT 6  | 11                    |

TABLE 3-continued

| Targeting of individual oligonucleotides<br>to specific variants of STAT 6 |                        |                |         |                       |
|----------------------------------------------------------------------------|------------------------|----------------|---------|-----------------------|
| ISIS #                                                                     | OLIGO<br>SEQ ID<br>NO. | TARGET<br>SITE | VARIANT | VARIANT SEQ<br>ID NO. |
| 195438                                                                     | 63                     | 2925           | STAT 6c | 137                   |
| 195438                                                                     | 63                     | 2734           | STAT 6b | 138                   |
| 195439                                                                     | 64                     | 23             | STAT 6  | 11                    |
| 195440                                                                     | 65                     | 38             | STAT 6  | 11                    |
| 195441                                                                     | 66                     | 3185           | STAT 6  | 11                    |
| 195441                                                                     | 66                     | 3052           | STAT 6c | 137                   |
| 195441                                                                     | 66                     | 2861           | STAT 6b | 138                   |
| 195442                                                                     | 67                     | 3230           | STAT 6  | 11                    |
| 195442                                                                     | 67                     | 3097           | STAT 6c | 137                   |
| 195442                                                                     | 67                     | 2906           | STAT 6b | 138                   |
| 195443                                                                     | 68                     | 3531           | STAT 6  | 11                    |
| 195443                                                                     | 68                     | 3398           | STAT 6c | 137                   |
| 195443                                                                     | 68                     | 3207           | STAT 6b | 138                   |
| 195444                                                                     | 69                     | 3539           | STAT 6  | 11                    |
| 195444                                                                     | 69                     | 3406           | STAT 6c | 137                   |
| 195444                                                                     | 69                     | 3215           | STAT 6b | 138                   |
| 195445                                                                     | 70                     | 3585           | STAT 6  | 11                    |
| 195445                                                                     | 70                     | 3452           | STAT 6c | 137                   |
| 195445                                                                     | 70                     | 3261           | STAT 6b | 138                   |
| 195446                                                                     | 71                     | 3599           | STAT 6  | 11                    |
| 195446                                                                     | 71                     | 3466           | STAT 6c | 137                   |
| 195446                                                                     | 71                     | 3275           | STAT 6b | 138                   |
| 195447                                                                     | 72                     | 3629           | STAT 6  | 11                    |
| 195447                                                                     | 72                     | 3496           | STAT 6c | 137                   |

TABLE 3-continued

| Targeting of individual oligonucleotides<br>to specific variants of STAT 6 |                        |                |         |                       |
|----------------------------------------------------------------------------|------------------------|----------------|---------|-----------------------|
| ISIS #                                                                     | OLIGO<br>SEQ ID<br>NO. | TARGET<br>SITE | VARIANT | VARIANT SEQ<br>ID NO. |
| 195447                                                                     | 72                     | 3305           | STAT 6b | 138                   |
| 195448                                                                     | 73                     | 3779           | STAT 6  | 11                    |
| 195448                                                                     | 73                     | 3646           | STAT 6c | 137                   |
| 195448                                                                     | 73                     | 3455           | STAT 6b | 138                   |
| 195449                                                                     | 74                     | 3835           | STAT 6  | 11                    |
| 195449                                                                     | 74                     | 3702           | STAT 6c | 137                   |
| 195449                                                                     | 74                     | 3511           | STAT 6b | 138                   |
| 195453                                                                     | 78                     | 1567           | STAT 6  | 11                    |
| 195453                                                                     | 78                     | 1517           | STAT 6c | 137                   |
| 195453                                                                     | 78                     | 1242           | STAT 6b | 138                   |
| 195455                                                                     | 80                     | 624            | STAT 6d | 13                    |
| 195456                                                                     | 81                     | 90             | STAT 6e | 14                    |
| 195458                                                                     | 83                     | 9              | STAT 6d | 13                    |
| 195459                                                                     | 84                     | 148            | STAT 6d | 13                    |
| 195460                                                                     | 85                     | 654            | STAT 6d | 13                    |
| 195461                                                                     | 86                     | 66             | STAT 6e | 14                    |

[0223]

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 138

<210> SEQ ID NO 1  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 1

tccgtcatcg ctcctcaggg 20

<210> SEQ ID NO 2  
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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 2

gtgcgcgcga gcccgaaatc 20

<210> SEQ ID NO 3  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 3

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<210> SEQ ID NO 4
<211> LENGTH: 3046
<212> TYPE: DNA
<213> ORGANISM: H. sapiens
<220> FEATURE:
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (166)...(2709)

<400> SEQUENCE: 4

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tgaactcgct ggacagagct acagacctat ggggcctgga agtgcccgcct gagaaaggga      120
gaagacagca gaggggttgc cgaggcaacc tccaagtccc agatc atg tct ctg tgg      177
                               Met Ser Leu Trp
                               1

ggt ctg gtc tcc aag atg ccc cca gaa aaa gtg cag cgg ctc tat gtc      225
Gly Leu Val Ser Lys Met Pro Pro Glu Lys Val Gln Arg Leu Tyr Val
  5              10              15              20

gac ttt ccc caa cac ctg cgg cat ctt ctg ggt gac tgg ctg gag agc      273
Asp Phe Pro Gln His Leu Arg His Leu Leu Gly Asp Trp Leu Glu Ser
              25              30              35

cag ccc tgg gag ttc ctg gtc ggc tcc gac gcc ttc tgc tgc aac ttg      321
Gln Pro Trp Glu Phe Leu Val Gly Ser Asp Ala Phe Cys Cys Asn Leu
              40              45              50

gct agt gcc cta ctt tca gac act gtc cag cac ctt cag gcc tcg gtg      369
Ala Ser Ala Leu Leu Ser Asp Thr Val Gln His Leu Gln Ala Ser Val
  55              60              65

gga gag cag ggg gag ggg agc acc atc ttg caa cac atc agc acc ctt      417
Gly Glu Gln Gly Glu Gly Ser Thr Ile Leu Gln His Ile Ser Thr Leu
  70              75              80

gag agc ata tat cag agg gac ccc ctg aag ctg gtg gcc act ttc aga      465
Glu Ser Ile Tyr Gln Arg Asp Pro Leu Lys Leu Val Ala Thr Phe Arg
  85              90              95              100

caa ata ctt caa gga gag aaa aaa gct gtt atg gaa cag ttc cgc cac      513
Gln Ile Leu Gln Gly Glu Lys Lys Ala Val Met Glu Gln Phe Arg His
  105              110              115

ttg cca atg cct ttc cac tgg aag cag gaa gaa ctc aag ttt aag aca      561
Leu Pro Met Pro Phe His Trp Lys Gln Glu Glu Leu Lys Phe Lys Thr
  120              125              130

ggc ttg cgg agg ctg cag cac cga gta ggg gag atc cac ctt ctc cga      609
Gly Leu Arg Arg Leu Gln His Arg Val Gly Glu Ile His Leu Leu Arg
  135              140              145

gaa gcc ctg cag aag ggg gct gag gct ggc caa gtg tct ctg cac agc      657
Glu Ala Leu Gln Lys Gly Ala Glu Ala Gly Gln Val Ser Leu His Ser
  150              155              160

ttg ata gaa act cct gct aat ggg act ggg cca agt gag gcc ctg gcc      705
Leu Ile Glu Thr Pro Ala Asn Gly Thr Gly Pro Ser Glu Ala Leu Ala
  165              170              175              180

atg cta ctg cag gag acc act gga gag cta gag gca gcc aaa gcc cta      753
Met Leu Leu Gln Glu Thr Thr Gly Glu Leu Glu Ala Ala Lys Ala Leu
  185              190              195

gtg ctg aag agg atc cag att tgg aaa cgg cag cag cag ctg gca ggg      801
Val Leu Lys Arg Ile Gln Ile Trp Lys Arg Gln Gln Gln Leu Ala Gly
  200              205              210

aat ggc gca ccg ttt gag gag agc ctg gcc cca ctc cag gag agg tgt      849
Asn Gly Ala Pro Phe Glu Glu Ser Leu Ala Pro Leu Gln Glu Arg Cys
  215              220              225

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|                                                                                                                                                       |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| gaa agc ctg gtg gac att tat tcc cag cta cag cag gag gta ggg gcg<br>Glu Ser Leu Val Asp Ile Tyr Ser Gln Leu Gln Gln Glu Val Gly Ala<br>230 235 240     | 897  |
| gct ggt ggg gag ctt gag ccc aag acc cgg gca tcg ctg act ggc cgg<br>Ala Gly Gly Glu Leu Glu Pro Lys Thr Arg Ala Ser Leu Thr Gly Arg<br>245 250 255 260 | 945  |
| ctg gat gaa gtc ctg aga acc ctc gtc acc agt tgc ttc ctg gtg gag<br>Leu Asp Glu Val Leu Arg Thr Leu Val Thr Ser Cys Phe Leu Val Glu<br>265 270 275     | 993  |
| aag cag ccc ccc cag gta ctg aag act cag acc aag ttc cag gct gga<br>Lys Gln Pro Pro Gln Val Leu Lys Thr Gln Thr Lys Phe Gln Ala Gly<br>280 285 290     | 1041 |
| gtt cga ttc ctg ttg ggc ttg agg ttc ctg ggg gcc cca gcc aag cct<br>Val Arg Phe Leu Leu Gly Leu Arg Phe Leu Gly Ala Pro Ala Lys Pro<br>295 300 305     | 1089 |
| ccg ctg gtc agg gcc gac atg gtg aca gag aag cag gcg cgg gag ctg<br>Pro Leu Val Arg Ala Asp Met Val Thr Glu Lys Gln Ala Arg Glu Leu<br>310 315 320     | 1137 |
| agt gtg cct cag ggt cct ggg gct gga gca gaa agc act gga gaa atc<br>Ser Val Pro Gln Gly Pro Gly Ala Gly Ala Glu Ser Thr Gly Glu Ile<br>325 330 335 340 | 1185 |
| atc aac aac act gtg ccc ttg gag aac agc att cct ggg aac tgc tgc<br>Ile Asn Asn Thr Val Pro Leu Glu Asn Ser Ile Pro Gly Asn Cys Cys<br>345 350 355     | 1233 |
| tct gcc ctg ttc aag aac ctg ctt ctc aag aag atc aag cgg tgt gag<br>Ser Ala Leu Phe Lys Asn Leu Leu Leu Lys Lys Ile Lys Arg Cys Glu<br>360 365 370     | 1281 |
| cgg aag ggc act gag tct gtc aca gag gag aag tgc gct gtg ctc ttc<br>Arg Lys Gly Thr Glu Ser Val Thr Glu Glu Lys Cys Ala Val Leu Phe<br>375 380 385     | 1329 |
| tct gcc agc ttc aca ctt ggc ccc ggc aaa ctc ccc atc cag ctc cag<br>Ser Ala Ser Phe Thr Leu Gly Pro Gly Lys Leu Pro Ile Gln Leu Gln<br>390 395 400     | 1377 |
| gcc ctg tct ctg ccc ctg gtg gtc atc gtc cat ggc aac caa gac aac<br>Ala Leu Ser Leu Pro Leu Val Val Ile Val His Gly Asn Gln Asp Asn<br>405 410 415 420 | 1425 |
| aat gcc aaa gcc act atc ctg tgg gac aat gcc ttc tct gag atg gac<br>Asn Ala Lys Ala Thr Ile Leu Trp Asp Asn Ala Phe Ser Glu Met Asp<br>425 430 435     | 1473 |
| cgc gtg ccc ttt gtg gtg gct gag cgg gtg ccc tgg gag aag atg tgt<br>Arg Val Pro Phe Val Val Ala Glu Arg Val Pro Trp Glu Lys Met Cys<br>440 445 450     | 1521 |
| gaa act ctg aac ctg aag ttc atg gct gag gtg ggg acc aac cgg ggg<br>Glu Thr Leu Asn Leu Lys Phe Met Ala Glu Val Gly Thr Asn Arg Gly<br>455 460 465     | 1569 |
| ctg ctc cca gag cac ttc ctc ttc ctg gcc cag aag atc ttc aat gac<br>Leu Leu Pro Glu His Phe Leu Phe Leu Ala Gln Lys Ile Phe Asn Asp<br>470 475 480     | 1617 |
| aac agc ctc agt atg gag gcc ttc cag cac cgt tct gtg tcc tgg tcg<br>Asn Ser Leu Ser Met Glu Ala Phe Gln His Arg Ser Val Ser Trp Ser<br>485 490 495 500 | 1665 |
| cag ttc aac aag gag atc ctg ctg ggc cgt ggc ttc acc ttt tgg cag<br>Gln Phe Asn Lys Glu Ile Leu Leu Gly Arg Gly Phe Thr Phe Trp Gln<br>505 510 515     | 1713 |
| tgg ttt gat ggt gtc ctg gac ctc acc aaa cgc tgt ctc cgg agc tac<br>Trp Phe Asp Gly Val Leu Asp Leu Thr Lys Arg Cys Leu Arg Ser Tyr<br>520 525 530     | 1761 |

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|            |         |        |        |        |        |        |        |      |
|------------|---------|--------|--------|--------|--------|--------|--------|------|
| tggtctgac  | cggtctg | atcatt | ggcttc | atcagc | aaacag | tactgt | act    | 1809 |
| TrpSer     | AspArg  | LeuIle | IleGly | PheIle | SerLys | GlnTyr | ValThr |      |
| 535        |         |        | 540    |        |        | 545    |        |      |
| agccttctt  | ctcaat  | gagccc | gacgga | acc    | tttctc | ctcgcg | ttcagc | 1857 |
| SerLeu     | LeuLeu  | AsnGlu | ProAsp | GlyThr | PheLeu | LeuArg | PheSer |      |
| 550        |         | 555    |        |        | 560    |        |        |      |
| gactcatgag | attggg  | ggc    | atcacc | attgcc | catgtc | atc    | cgggcg | 1905 |
| AspSer     | GluIle  | GlyGly | IleThr | IleAla | HisVal | IleArg | GlyGln |      |
| 565        |         | 570    |        |        | 575    |        | 580    |      |
| gatggctct  | ccacag  | atagag | aac    | atccag | cca    | ttctct | gccaaa | 1953 |
| AspGly     | SerPro  | GlnIle | GluAsn | IleGln | ProPhe | SerAla | LysAsp |      |
|            |         | 585    |        | 590    |        |        | 595    |      |
| ctgtccatt  | cgctca  | ctggg  | gac    | cga    | atc    | cgggat | cttgct | 2001 |
| LeuSer     | IleArg  | SerLeu | GlyAsp | ArgIle | ArgAsp | LeuAla | GlnLeu |      |
|            | 600     |        |        | 605    |        |        | 610    |      |
| aaa        | aatctc  | tatccc | aagaag | ccc    | aag    | gatgag | gctttc | 2049 |
| LysAsn     | LeuTyr  | ProLys | LysLys | ProLys | AspGlu | AlaPhe | ArgSer |      |
| 615        |         |        | 620    |        |        |        | 625    |      |
| tac        | aagcct  | gaacag | atgggt | aag    | gatggc | agg    | ggttat | 2097 |
| TyrLys     | ProGlu  | GlnMet | GlyLys | AspGly | ArgGly | TyrVal | ProAla |      |
| 630        |         |        | 635    |        |        | 640    |        |      |
| acc        | atcaag  | atgacc | gtggaa | agg    | gac    | caa    | cca    | 2145 |
| ThrIle     | LysMet  | ThrVal | GluArg | AspGln | ProLeu | ProThr | ProGlu |      |
| 645        |         | 650    |        |        | 655    |        | 660    |      |
| ctc        | cagatg  | cctacc | atggtg | ccttct | tattac | gtc    | ctt    | 2193 |
| LeuGln     | MetPro  | ThrMet | ValPro | SerTyr | AspLeu | GlyMet | AlaPro |      |
|            | 665     |        |        | 670    |        |        | 675    |      |
| gat        | tcc     | tccatg | agc    | atgcag | cttggc | cca    | gat    | 2241 |
| AspSer     | SerMet  | SerMet | GlnLeu | GlyPro | AspMet | ValPro | GlnVal |      |
|            | 680     |        |        | 685    |        |        | 690    |      |
| tac        | cca     | cca    | cactct | cactcc | atcccc | cggtat | caaggc | 2289 |
| TyrPro     | ProHis  | SerHis | SerIle | ProPro | TyrGln | GlyLeu | SerPro |      |
|            | 695     |        | 700    |        |        | 705    |        |      |
| gaa        | gaa     | tca    | gtcaac | gtgttg | tca    | gcc    | ttc    | 2337 |
| GluGlu     | SerVal  | AsnVal | LeuSer | AlaPhe | GlnGlu | ProHis | LeuGln |      |
| 710        |         |        | 715    |        |        | 720    |        |      |
| atg        | ccc     | ccc    | agc    | ctgggc | cag    | atg    | agc    | 2385 |
| MetPro     | ProSer  | LeuGly | GlnMet | SerLeu | ProPhe | AspGln | ProHis |      |
| 725        |         |        | 730    |        |        | 735    | 740    |      |
| ccc        | cag     | ggc    | ctg    | ctgcg  | tgc    | cag    | cct    | 2433 |
| ProGln     | GlyLeu  | LeuPro | CysGln | ProGln | GluHis | AlaVal | SerSer |      |
|            | 745     |        |        | 750    |        |        | 755    |      |
| cct        | gac     | ccc    | ctg    | ctctgc | tca    | gat    | gtg    | 2481 |
| ProAsp     | ProLeu  | LeuCys | SerAsp | ValThr | MetVal | GluAsp | SerCys |      |
|            | 760     |        |        | 765    |        |        | 770    |      |
| ctg        | agc     | cag    | cca    | gtgaca | gcgttt | cctcag | ggc    | 2529 |
| LeuSer     | GlnPro  | ValThr | AlaPhe | ProGln | GlyThr | TrpIle | GlyGlu |      |
|            | 775     |        | 780    |        |        | 785    |        |      |
| gac        | ata     | ttc    | cct    | cctctg | ctg    | cctccc | act    | 2577 |
| AspIle     | PhePro  | ProPro | LeuLeu | ProPro | ThrGlu | GlnAsp | LeuThr |      |
|            | 790     |        | 795    |        |        | 800    |        |      |
| ctt        | ctc     | ctg    | gag    | ggg    | caa    | ggg    | gag    | 2625 |
| LeuLeu     | LeuGlu  | GlyGly | GlnGlu | SerGly | GlyGly | SerLeu | GlyAla |      |
| 805        |         |        | 810    |        | 815    |        | 820    |      |
| cag        | ccc     | ctc    | ctg    | cag    | ccc    | tcc    | cac    | 2673 |
| GlnPro     | LeuLeu  | GlnPro | SerHis | TyrGly | GlnSer | GlyIle | SerMet |      |
|            | 825     |        |        | 830    |        |        | 835    |      |

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tcc cac atg gac cta agg gcc aac ccc agt tgg tga tcccagctgg 2719  
Ser His Met Asp Leu Arg Ala Asn Pro Ser Trp  
840 845

agggagaacc caaagagaca gctcttctac tacccccaca gacctgctct ggacacttgc 2779

tcatgccctg ccaagcagca gatggggagg gtgcctctct atccccacct actcctgggt 2839

caggaggaaa agactaacag gagaatgcac agtgggtgga gccaatccac tccttccttt 2899

ctatcattcc cctgcccacc tccttcacgc actgactgga agggaagtgc aggctctgag 2959

acacgcccca acatgctgc acctgcagcg cgcacacgca cgcacacaca catacagagc 3019

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<210> SEQ ID NO 5  
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<400> SEQUENCE: 5

cctaaacgctg tctccgga 18

<210> SEQ ID NO 6  
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<220> FEATURE:  
<223> OTHER INFORMATION: PCR Primer

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gctagtaacg tactgtttgc tgatgaa 27

<210> SEQ ID NO 7  
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<223> OTHER INFORMATION: PCR Probe

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<212> TYPE: DNA  
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<212> TYPE: DNA  
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<220> FEATURE:  
<223> OTHER INFORMATION: PCR Primer

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<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: PCR Probe

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caagcttccc gttctcagcc 20

<210> SEQ ID NO 11  
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SEQUENCE: 11

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atttttcttt ttggtggtgg tgggtgaagg ggggaggtgc tagcagggcc agccttgaac 180

tcgctggaca gagctacaga cctatggggc ctggaagtgc ccgctgagaa agggagaaga 240

cagcagaggg gttgccgagg caacctccaa gtcccagatc atg tct ctg tgg ggt 295  
Met Ser Leu Trp Gly  
1 5

ctg gtc tcc aag atg ccc cca gaa aaa gtg cag cgg ctc tat gtc gac 343  
Leu Val Ser Lys Met Pro Pro Glu Lys Val Gln Arg Leu Tyr Val Asp  
10 15 20

ttt ccc caa cac ctg cgg cat ctt ctg ggt gac tgg ctg gag agc cag 391  
Phe Pro Gln His Leu Arg His Leu Leu Gly Asp Trp Leu Glu Ser Gln  
25 30 35

ccc tgg gag ttc ctg gtc ggc tcc gac gcc ttc tgc tgc aac ttg gct 439  
Pro Trp Glu Phe Leu Val Gly Ser Asp Ala Phe Cys Cys Asn Leu Ala  
40 45 50

agt gcc cta ctt tca gac act gtc cag cac ctt cag gcc tcg gtg gga 487  
Ser Ala Leu Leu Ser Asp Thr Val Gln His Leu Gln Ala Ser Val Gly  
55 60 65

gag cag ggg gag ggg agc acc atc ttg caa cac atc agc acc ctt gag 535  
Glu Gln Gly Glu Gly Ser Thr Ile Leu Gln His Ile Ser Thr Leu Glu  
70 75 80 85

agc ata tat cag agg gac ccc ctg aag ctg gtg gcc act ttc aga caa 583  
Ser Ile Tyr Gln Arg Asp Pro Leu Lys Leu Val Ala Thr Phe Arg Gln  
90 95 100

ata ctt caa gga gag aaa aaa gct gtt atg gaa cag ttc cgc cac ttg 631  
Ile Leu Gln Gly Glu Lys Lys Ala Val Met Glu Gln Phe Arg His Leu  
105 110 115

cca atg cct ttc cac tgg aag cag gaa gaa ctc aag ttt aag aca ggc 679  
Pro Met Pro Phe His Trp Lys Gln Glu Glu Leu Lys Phe Lys Thr Gly  
120 125 130

ttg cgg agg ctg cag cac cga gta ggg gag atc cac ctt ctc cga gaa 727  
Leu Arg Arg Leu Gln His Arg Val Gly Glu Ile His Leu Leu Arg Glu  
135 140 145

gcc ctg cag aag ggg gct gag gct ggc caa gtg tct ctg cac agc ttg 775  
Ala Leu Gln Lys Gly Ala Glu Ala Gly Gln Val Ser Leu His Ser Leu  
150 155 160 165

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|                                                                                                                                                       |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| ata gaa act cct gct aat ggg act ggg cca agt gag gcc ctg gcc atg<br>Ile Glu Thr Pro Ala Asn Gly Thr Gly Pro Ser Glu Ala Leu Ala Met<br>170 175 180     | 823  |
| cta ctg cag gag acc act gga gag cta gag gca gcc aaa gcc cta gtg<br>Leu Leu Gln Glu Thr Thr Gly Glu Leu Glu Ala Ala Lys Ala Leu Val<br>185 190 195     | 871  |
| ctg aag agg atc cag att tgg aaa cgg cag cag cag ctg gca ggg aat<br>Leu Lys Arg Ile Gln Ile Trp Lys Arg Gln Gln Gln Leu Ala Gly Asn<br>200 205 210     | 919  |
| ggc gca ccg ttt gag gag agc ctg gcc cca ctc cag gag agg tgt gaa<br>Gly Ala Pro Phe Glu Glu Ser Leu Ala Pro Leu Gln Glu Arg Cys Glu<br>215 220 225     | 967  |
| agc ctg gtg gac att tat tcc cag cta cag cag gag gta ggg gcg gct<br>Ser Leu Val Asp Ile Tyr Ser Gln Leu Gln Gln Glu Val Gly Ala Ala<br>230 235 240 245 | 1015 |
| ggg ggg gag ctt gag ccc aag acc cgg gca tcg ctg act ggc cgg ctg<br>Gly Gly Glu Leu Glu Pro Lys Thr Arg Ala Ser Leu Thr Gly Arg Leu<br>250 255 260     | 1063 |
| gat gaa gtc ctg aga acc ctc gtc acc agt tgc ttc ctg gtg gag aag<br>Asp Glu Val Leu Arg Thr Leu Val Thr Ser Cys Phe Leu Val Glu Lys<br>265 270 275     | 1111 |
| cag ccc ccc cag gta ctg aag act cag acc aag ttc cag gct gga gtt<br>Gln Pro Pro Gln Val Leu Lys Thr Gln Thr Lys Phe Gln Ala Gly Val<br>280 285 290     | 1159 |
| cga ttc ctg ttg ggc ttg agg ttc ctg ggg gcc cca gcc aag cct ccg<br>Arg Phe Leu Leu Gly Leu Arg Phe Leu Gly Ala Pro Ala Lys Pro Pro<br>295 300 305     | 1207 |
| ctg gtc agg gcc gac atg gtg aca gag aag cag gcg cgg gag ctg agt<br>Leu Val Arg Ala Asp Met Val Thr Glu Lys Gln Ala Arg Glu Leu Ser<br>310 315 320 325 | 1255 |
| gtg cct cag ggt cct ggg gct gga gca gaa agc act gga gaa atc atc<br>Val Pro Gln Gly Pro Gly Ala Gly Ala Glu Ser Thr Gly Glu Ile Ile<br>330 335 340     | 1303 |
| aac aac act gtg ccc ttg gag aac agc att cct ggg aac tgc tgc tct<br>Asn Asn Thr Val Pro Leu Glu Asn Ser Ile Pro Gly Asn Cys Cys Ser<br>345 350 355     | 1351 |
| gcc ctg ttc aag aac ctg ctt ctc aag aag atc aag cgg tgt gag cgg<br>Ala Leu Phe Lys Asn Leu Leu Lys Lys Ile Lys Arg Cys Glu Arg<br>360 365 370         | 1399 |
| aag ggc act gag tct gtc aca gag gag aag tgc gct gtg ctc ttc tct<br>Lys Gly Thr Glu Ser Val Thr Glu Glu Lys Cys Ala Val Leu Phe Ser<br>375 380 385     | 1447 |
| gcc agc ttc aca ctt ggc ccc ggc aaa ctc ccc atc cag ctc cag gcc<br>Ala Ser Phe Thr Leu Gly Pro Gly Lys Leu Pro Ile Gln Leu Gln Ala<br>390 395 400 405 | 1495 |
| ctg tct ctg ccc ctg gtg gtc atc gtc cat ggc aac caa gac aac aat<br>Leu Ser Leu Pro Leu Val Val Ile Val His Gly Asn Gln Asp Asn Asn<br>410 415 420     | 1543 |
| gcc aaa gcc act atc ctg tgg tac aat gcc ttc tct gag atg gac cgc<br>Ala Lys Ala Thr Ile Leu Trp Tyr Asn Ala Phe Ser Glu Met Asp Arg<br>425 430 435     | 1591 |
| gtg ccc ttt gtg gtg gct gag cgg gtg ccc tgg gag aag atg tgt gaa<br>Val Pro Phe Val Val Ala Glu Arg Val Pro Trp Glu Lys Met Cys Glu<br>440 445 450     | 1639 |
| act ctg aac ctg aag ttc atg gct gag gtg ggg acc aac cgg ggg ctg<br>Thr Leu Asn Leu Lys Phe Met Ala Glu Val Gly Thr Asn Arg Gly Leu<br>455 460 465     | 1687 |

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|                                                                     |                                             |      |
|---------------------------------------------------------------------|---------------------------------------------|------|
| ctc cca gag cac ttc                                                 | ctc ttc ctg gcc cag aag atc ttc aat gac aac | 1735 |
| Leu Pro Glu His Phe                                                 | Leu Phe Leu Ala Gln Lys Ile Phe Asn Asp Asn |      |
| 470                                                                 | 475 480 485                                 |      |
| agc ctc agt atg gag gcc ttc cag cac cgt tct gtg tcc tgg tcg cag     | 1783                                        |      |
| Ser Leu Ser Ser Met Glu Ala Phe Gln His Arg Ser Val Ser Trp Ser Gln |                                             |      |
|                                                                     | 490 495 500                                 |      |
| ttc aac aag gag atc ctg ctg ggc cgt ggc ttc acc ttt tgg cag tgg     | 1831                                        |      |
| Phe Asn Lys Glu Ile Leu Leu Gly Arg Gly Phe Thr Phe Trp Gln Trp     |                                             |      |
|                                                                     | 505 510 515                                 |      |
| ttt gat ggt gtc ctg gac ctc acc aaa cgc tgt ctc cgg agc tac tgg     | 1879                                        |      |
| Phe Asp Gly Val Leu Asp Leu Thr Lys Arg Cys Leu Arg Ser Tyr Trp     |                                             |      |
|                                                                     | 520 525 530                                 |      |
| tct gac cgg ctg atc att ggc ttc atc agc aaa cag tac gtt act agc     | 1927                                        |      |
| Ser Asp Arg Leu Ile Ile Gly Phe Ile Ser Lys Gln Tyr Val Thr Ser     |                                             |      |
|                                                                     | 535 540 545                                 |      |
| ctt ctt ctc aat gag ccc gac gga acc ttt ctc ctc cgc ttc agc gac     | 1975                                        |      |
| Leu Leu Leu Asn Glu Pro Asp Gly Thr Phe Leu Leu Arg Phe Ser Asp     |                                             |      |
|                                                                     | 550 555 560 565                             |      |
| tca gag att ggg ggc atc acc att gcc cat gtc atc cgg ggc cag gat     | 2023                                        |      |
| Ser Glu Ile Gly Gly Ile Thr Ile Ala His Val Ile Arg Gly Gln Asp     |                                             |      |
|                                                                     | 570 575 580                                 |      |
| ggc tct cca cag ata gag aac atc cag cca ttc tct gcc aaa gac ctg     | 2071                                        |      |
| Gly Ser Pro Gln Ile Glu Asn Ile Gln Pro Phe Ser Ala Lys Asp Leu     |                                             |      |
|                                                                     | 585 590 595                                 |      |
| tcc att cgc tca ctg ggg gac cga atc cgg gat ctt gct cag ctc aaa     | 2119                                        |      |
| Ser Ile Arg Ser Leu Gly Asp Arg Ile Arg Asp Leu Ala Gln Leu Lys     |                                             |      |
|                                                                     | 600 605 610                                 |      |
| aat ctc tat ccc aag aag ccc aag gat gag gct ttc cgg agc cac tac     | 2167                                        |      |
| Asn Leu Tyr Pro Lys Lys Pro Lys Asp Glu Ala Phe Arg Ser His Tyr     |                                             |      |
|                                                                     | 615 620 625                                 |      |
| aag cct gaa cag atg ggt aag gat ggc agg ggt tat gtc cca gct acc     | 2215                                        |      |
| Lys Pro Glu Gln Met Gly Lys Asp Gly Arg Gly Tyr Val Pro Ala Thr     |                                             |      |
|                                                                     | 630 635 640 645                             |      |
| atc aag atg acc gtg gaa agg gac caa cca ctt cct acc cca gag ctc     | 2263                                        |      |
| Ile Lys Met Thr Val Glu Arg Asp Gln Pro Leu Pro Thr Pro Glu Leu     |                                             |      |
|                                                                     | 650 655 660                                 |      |
| cag atg cct acc atg gtg cct tct tat gac ctt gga atg gcc ctg att     | 2311                                        |      |
| Gln Met Pro Thr Met Val Pro Ser Tyr Asp Leu Gly Met Ala Leu Ile     |                                             |      |
|                                                                     | 665 670 675                                 |      |
| cct cca tga gcatagcagct tggcccagat atggtgcccc aggtgtaccc accacactct | 2370                                        |      |
| Pro Pro                                                             |                                             |      |
| cactccatcc ccccgatca aggcctctcc ccagaagaat cagtaacgt gttgtcagcc     | 2430                                        |      |
| ttccaggagc ctcacctgca gatgcccccc agcctgggcc agatgagcct gccctttgac   | 2490                                        |      |
| cagcctcacc cccagggcct gctgcctgac cagcctcagg agcatgctgt gtccagccct   | 2550                                        |      |
| gacccctcgc tctgctcaga tgtgacctg gtggaagaca gctgcctgag ccagccagt     | 2610                                        |      |
| acagcgtttc ctcagggcac ttgatttggt gaagacatat tccctcctct gctgcctccc   | 2670                                        |      |
| actgaacagg acctcactaa gcttctcctg gaggggcaag gggagtcggg gggagggtcc   | 2730                                        |      |
| ttggggggcac agccctcct gcagccctcc cactatgggc aatctgggat ctcaatgtcc   | 2790                                        |      |
| cacatggacc taagggccaa cccagttg tgatcccagc tggagggaga acccaaagag     | 2850                                        |      |
| acagctcttc tactaccccc acagacctgc totggacact tgctcatgcc ctgccaagca   | 2910                                        |      |
| gcagatgggg aggtgcccct cctatccca cctactcctg ggtcaggagg aaaagactaa    | 2970                                        |      |

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|                                                                   |      |
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| acctccttcc agcactgact ggaagggaag ttcaggctct gagacacgcc ccaacatgcc | 3090 |
| tgcacctgca gcgcgcacac gcacgcacac acacatacag agctctctga gggatgatgg | 3150 |
| gctgagcagg aggggggctg ggtaagagca caggtaggg catggaaggc ttctccgccc  | 3210 |
| attctgaccc agggcctagg acggataggc aggaacatac agacacattt aactagagg  | 3270 |
| ccagggatag aggatattgg gtctcagccc taggggaatg ggaagcagct caagggaccc | 3330 |
| tgggtgggag cataggagg gtctggacat gtggttacta gtacaggttt tgcctgatt   | 3390 |
| aaaaaatctc ccaaagcccc aaattcctgt tagccagggtg gaggcttctg atactgtat | 3450 |
| gagactatgc aaagtacaa gggctgagat tcttcgtgta tagctgtgtg aacgtgtatg  | 3510 |
| tacctaggat atgttaaatg tatagctggc acctagtgtg catgaccaca tagaacatgt | 3570 |
| gtctatctgc ttttgcctac gtgacaacac aaatttgga gggtagagca ctgcacagaa  | 3630 |
| gacagcagca agtggtctgg cctctctgac atatgctaac ccccaatac tctgaatttg  | 3690 |
| gagtctgact gtgccaagt gggccaagt ggctgtgaca tctacgtatg gctccacacc   | 3750 |
| tccaatgctg cctgggagcc agggtagag tctgggtcca ggcctggcca tgtggccctc  | 3810 |
| cagtgtatga gagggccctg cctgctgcat cttttctgtt gcccaccca ccgccagctt  | 3870 |
| cccttcactc ccctatccca ttctccctct caaggcaggg gtcatagatc ctaagccata | 3930 |
| aaataaatgt tattccaaa taaaaaaaa aaaaaaaaa a                        | 3971 |

&lt;210&gt; SEQ ID NO 12

&lt;211&gt; LENGTH: 16500

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: H. sapiens

&lt;220&gt; FEATURE:

&lt;400&gt; SEQUENCE: 12

|                                                                    |      |
|--------------------------------------------------------------------|------|
| tcccccaag cctggctcca aggcctggac ccagtcctg atccccacg tgttcccca      | 60   |
| ctcggcacag gaggcacaca tattcaccac actttcttcc tcttcctcct ccagccact   | 120  |
| ttctcttctc tgtgtcgtca gagctccagg gagggacctg ggtagaagga gaagccgaa   | 180  |
| acagcgggct ggggcagcca ctgcttacac tgaagaggga ggacgggaga ggagtgtgtg  | 240  |
| tgtgtgtgtg tgtgtgtgta tgtatgtgtg tgctttatct tatttttctt ttggtgtgtg  | 300  |
| gtggtggaag gggggagggt ctagcagggc cagccttga ctcgctggac agagctacag   | 360  |
| acctatgggg cctggaagtg cccgctgaga aagggaaga acagcagagg ggttgccgag   | 420  |
| gtgaggggtt gcctccgagg tgggtgcggg ggcctctatg agtgcatggg ggtggattcg  | 480  |
| tggggggagc tctcgggatc ctcccctggc tgggtggatg gtcccaaga gatggttca    | 540  |
| gctagtgttg gtggtgggtg gcaactgggt ttagcagttt cgaactcctg gaggaatctg  | 600  |
| ggaggggtcca ggcctcagta ctcccctccc ccattgggtc cgttttcaca gcctcaccac | 660  |
| tgcaccccca gggccatgga aagtcaggga aaggaggta aggagtgccc ctctgccctg   | 720  |
| agtcggggga agtgcccgcc cctccctgga aggttgatgc cagagggcag tggatccttg  | 780  |
| ttaaaccctc atctgcctc ccactaaagg ttcctgttca agggtgtggc tggggcgtga   | 840  |
| gcaagcccca gatgtagacc tcatgtgtgc ccagacgagg ggaatttcc ccctcaaac    | 900  |
| tgtccacgc ttggtgctg tagacgtga gatttcccag cggcggcgcc gagttaacc      | 960  |
| tcctcgtgct gaactggctc cactccccc cctgccccca ccgccacatt cagcattgg    | 1020 |

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|                                                                    |      |
|--------------------------------------------------------------------|------|
| gcaactcaga gaagctgttt taactttcga tcctgtggtc ccacaatcag aggactcggg  | 1080 |
| cagatagggg ttgagataag cgagtttagg ccaccaagcg ggcggacgag gatcccagac  | 1140 |
| cttgcgcttc ccttctgagt ttgggaggta aactggccc cggccctcac gccgtggctc   | 1200 |
| ctccctccct tccccttcaa ggggctgaag aaaaagtg cccctgtcct ggtcaagcca    | 1260 |
| atcgaccacg ccttggtatg ggttgggggtg gggagaaatg tgcctcctg atggctgggg  | 1320 |
| aagaagaggg gttggatatt tctagccagg gccatgccag gaggctggtc actctgcaag  | 1380 |
| gggatgcaga ggaagcggg gccactcac tcagaggac ctttctctct tgggctagag     | 1440 |
| aaagccctat tggaggaacc tgagcaggag gggtaaggat tctgccttga ggagaaaaga  | 1500 |
| gctggggtaa gtgggcactg gaggaagag gggcatgaag gtcttgagc agaaacatcc    | 1560 |
| agagaaggga cctctccatt ttccatccct ctgagaggcc tgggagagggt gagaggctga | 1620 |
| acgtgcaaca ggaggacttg gggttactgg gtttggggag acctggggag ttgtcatccc  | 1680 |
| atcctctccc tcatctctgg gagagggata ttatgagaaa cgtgaactga gaggccctg   | 1740 |
| ggaaaccact ggttaccag tcctccctga acctggaaat ggggatgcaa cccctcttc    | 1800 |
| tacttccctg tccctcctc tcctttctac ctgtttctgt ctctcatctt tgccttctag   | 1860 |
| ccctccagct tcctctctct tctaggctct ttccctctag cttactaaac ccgccttttt  | 1920 |
| tccagtctct tccatctct tccttagttc tctctacttt ccttttccac ctctcctcct   | 1980 |
| tcaagtctcc tcccaccttc cccacttct taggatgatc agatttgccc ctggaaggga   | 2040 |
| tcctaacaac acagtgcgat ggttaatccc cactcagatt caaagcctgc tttccaaact  | 2100 |
| cacttactga gtggccttg gcagagtaga gaaactcctt aagcctcagt ttcttcatct   | 2160 |
| ataaaatggg atattatata ttttaaaaag tgcgtgagg cctgaaggag ataatacact   | 2220 |
| gagtgtaatg cctcatacac agtaagtgt taacaaatag tagctgttat tactctcca    | 2280 |
| tcctcttcat catctagcct tgtggtttct atttttatct tatttcattt atttatttat  | 2340 |
| ttatttttag acagagtctc tctctgtcgc ccaggctgga gtgcagtggc tcgatctctg  | 2400 |
| ctcactgcaa gctccgcccc ccaggttcac gccattctgt cacctcagcc tcccagtag   | 2460 |
| ctgggactac aggcgctcgc caccacgccc tgctaatttt gtttttgat ttttagtaga   | 2520 |
| gatggggttt cactgtgtta gccaggatgg tcttgatctc ctgacctcgt gatctgcccg  | 2580 |
| cctcgccctc ccaaagcgct gggattacag gcatgagcca ctgcgcctgg ccgagccttg  | 2640 |
| tggttttcaa attatctcat ggagtcctag aattttgaga ggtttgtcta gggatgcctt  | 2700 |
| tggcgctcag aggtggggag agggaagtag aagcagtcga gtttcaggct ttccatgctt  | 2760 |
| gctttcaaca gggcatcttc ggtttcgtac cttttatgta attgagattc cacagattaa  | 2820 |
| aagctgacat tgcctaccgc tttaaaaagt ttggaaagt ttccactcat ctaacactca   | 2880 |
| tattttatag atgagaagat cgaagcccac aaagggaagg ctctttgccc acagaaccag  | 2940 |
| agccaggctc agagctgcaa ctaaatcctc tgccactcta agagagctct cgctctactg  | 3000 |
| ccctgtctcc ctttgctcc ccatccctct ggctacagct cagctcttcc caccctgtg    | 3060 |
| tctatcactg aaggagtac cccatctca ggcattgact caggatgccc ctggtttaag    | 3120 |
| gtggctggc catgagtgtt ggtggggaca gtccctagga gggctatcta tgggaggtcc   | 3180 |
| ctggctgccc caggagatag gccaaagttt ttgggcaccc ctgagagtgg cttattttt   | 3240 |
| ctcctccagg caacctcaa gtccagatc atgtctctgt ggggtctggt ctocaagatg    | 3300 |

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|                                                                     |      |
|---------------------------------------------------------------------|------|
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| tcttccctaa ctcatcttcc ttctccttag atttttctcc cctcaccat ggattcagaa    | 3480 |
| cttgagacct gttattccat gtgtagtgc ctagatttag caggaggtct gtgccccatc    | 3540 |
| aagaccaggc tatgaatggt gacagatgga gaccccatct cttaggaggc tgagccgaag   | 3600 |
| aggagggggg tttgggctgg gacaaaggca cttctcataa cagctagaag actgggaac    | 3660 |
| aaggcgcatg ggtgaaagct acagagggcc tagatggaga ataaggagcg agaaaggaa    | 3720 |
| tgctgagctt ttggctgtgg ggtaaagggt caggagagct gaggaagccc tggcctgagg   | 3780 |
| tagcctcatc ctgatcttcc tgcaggaggt tcctggtcgg ctccgacgcc ttctgctgca   | 3840 |
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| tatagtatat gtgtgaatgc aggcctgggg attggaggga ttgaaggaca tgggtaagag   | 4200 |
| caaagctcac tgtttaccac cctcatttct gtagagcata tatcagaggg acccctgaa    | 4260 |
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| attgtgatat tccacctccc accccaactc aatccctga gactttggcc tgagccatga    | 4380 |
| caaactagaa agaatttgaa cctcagtaaa ggctcagtgt tctaggccca ggaatgacca   | 4440 |
| aaggaggttc ctagggtcag agtgaacccc aagtcaagct cagggaatct ttctatgagg   | 4500 |
| gactgaaggt aagaggccgg ggagaacaga gcaagggata aggagctgat tctgctagga   | 4560 |
| gcaaggtctt atctccacga tattccaaaa ggtcaggaa gactgccaaa ggggagaggg    | 4620 |
| gaacaagaaa acgctatctg cagagcagag agtggaggcc aggtatagag ggaatgagcag  | 4680 |
| agtgtttcac ttcttgcat ctgtccttcc tgtgtagtgc cgccacttgc caatgccttt    | 4740 |
| ccactggaag caggaagaac tcaagtttaa gacaggcttg cggaggctgc agcaccgagt   | 4800 |
| aggggagatc caccttctcc gagaagccct gcagaagggg gctgaggctg gccaaagtggtg | 4860 |
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| tgaacctcta atctgtctgc acccttgatt tctgccccca accctcagtg tctctgcaca   | 4980 |
| gcttgataga aactcctgct aatgggactg ggccaagtga ggtgagtaat gggctgacag   | 5040 |
| gtggagacct tggtaaaagt gcagctggag ggaatggaagc tagacctcag aaagacacag  | 5100 |
| gctgaagtag ggcaagggaa tgccagagga gtgagaaaaa gagccgtatc ccaggagctg   | 5160 |
| ggtgtggagg cagcgtgagg ccctggctca ggccctctc tgcccatagg ccctggccat    | 5220 |
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| ccagatttgg aaacggcagc agcagctggc agggaatggc gcaccgtttg aggagagcct   | 5340 |
| ggccccactc caggagaggt tgggctaggg ctgatgggga agagggggca agctgggggt   | 5400 |
| gggcagctga ccctgctgaa ggccctacag gtgagagaaa gaagccaggc gggagggcct   | 5460 |
| tggagtggac caagatgcat aaaagccagt tccagcgggg ctgtgcacac tgtcgttcag   | 5520 |
| gtcgcatcct gtacaagtgg gcctagtgga ggggcacaag cggggactca tccaaccacg   | 5580 |

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|            |            |             |            |             |            |      |
|------------|------------|-------------|------------|-------------|------------|------|
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| ggtgggaaca | ctttccaatt | tgccacaaa   | gactgtaagt | ggtggcagtt  | gtcctgggtg | 5700 |
| caagagccgt | cgggggagag | gcagctgggt  | ttccacagg  | ggtgtaggca  | ctgagaatga | 5760 |
| acctcccacc | cagaccctag | gccaacagat  | cacagaaccc | ccttcagccc  | aggtagcctg | 5820 |
| cagccacacc | cactaccacc | cccacttctc  | cacacatgat | agcctttctc  | cctgggtata | 5880 |
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| gcgggtgccc tgggagaaga tgtgtgaaac tctgaacctg aagttcatgg ctgaggtggg  | 420 |
| gaccaaccgg gggctgtcc cagagcactt cctcttcctg gcccagaaga tcttcaatga   | 480 |
| caacagcctc agtatggagg ccttcacgca cgttctgtg tcctggtcgc agttcaacaa   | 540 |
| ggagatcctg ctggccgtgg cttcaccttt tggcagtggg ttgatgggtg cctggacctc  | 600 |
| accaacgctg tctccggagc tactggtctg accggtgagt cccaccctg ggtagtctga   | 660 |
| gcagccatac accagtcacc tccatactca ctgccca                           | 697 |

<210> SEQ ID NO 14  
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<212> TYPE: DNA  
<213> ORGANISM: H. sapiens  
<220> FEATURE:  
<221> NAME/KEY: unsure  
<222> LOCATION: 58  
<223> OTHER INFORMATION: unknown

<400> SEQUENCE: 14

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| tggacagtgg gtaggtcagg ggcttagtgc ttatctgcag gaaggagggg tggcatcnac | 60  |
| ccttggtcag tcacatgtac ctccctccct cctccaggga ccaaccactt cctacccag  | 120 |
| agctccagat gcctaccatg gtgccttctt atgaccttgg aatggcccct gattcctcca | 180 |
| tgagcatgca gcttggccca gatatgggtg cccaggtgta cccaccacac tctcactcca | 240 |
| tcccccgta tcaaggcctc tccccagaag aatcagtcaa cgtgttgtca gccttcagg   | 300 |
| agcctcacct gcagatgccc cccagcctgg gccagatgag cctgcccttt gaccagcctc | 360 |
| acccccaggg cctgctgtcg tgccagcctc tggagcatgc tgtgtccagc cctgaccccc | 420 |
| tgc                                                               | 423 |

<210> SEQ ID NO 15  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 15

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| agtgagcgaa tggacaggtc | 20 |
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<210> SEQ ID NO 16  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 16

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|-----------------------|----|
| cgctgtcact ggctggctca | 20 |
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<210> SEQ ID NO 17  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 17

ttgatgatatt ctccagtgtc 20

<210> SEQ ID NO 18

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 18

aggacttcat ccagccggcc 20

<210> SEQ ID NO 19

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 19

cccaggaacc tcaagcccaa 20

<210> SEQ ID NO 20

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 20

gtcaccaga agatgccga 20

<210> SEQ ID NO 21

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 21

tttcacggt catcttgatg 20

<210> SEQ ID NO 22

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 22

aagatggtgc tcccctcccc 20

<210> SEQ ID NO 23

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 23

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gccgtttcca aatctggatc 20

<210> SEQ ID NO 24  
<211> LENGTH: 20  
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<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 24

ctttggctgc ctctagctct 20

<210> SEQ ID NO 25  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 25

gtttggtgag gtccaggaca 20

<210> SEQ ID NO 26  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 26

catctgcagg tgaggctcct 20

<210> SEQ ID NO 27  
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<212> TYPE: DNA  
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 27

tggcccttag gtccatgtgg 20

<210> SEQ ID NO 28  
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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 28

ctatctgtgg agagccatcc 20

<210> SEQ ID NO 29  
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<212> TYPE: DNA  
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 29

attgagaaga aggctagtaa 20

<210> SEQ ID NO 30

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<211> LENGTH: 20  
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<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 30  
  
gctgatgtgt tgcaagatgg 20  
  
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<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 31  
  
gccccatcac cctcagagag 20  
  
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<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
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ccctctgata tatgctctca 20  
  
<210> SEQ ID NO 33  
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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 33  
  
gaaggctagt aacgtactgt 20  
  
<210> SEQ ID NO 34  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 34  
  
gttcgctcgg gctcattgag 20  
  
<210> SEQ ID NO 35  
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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 35  
  
gtcactggct ggctcaggca 20  
  
<210> SEQ ID NO 36  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 36

ttcagagttt cacacatctt 20

<210> SEQ ID NO 37

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 37

caggcccat aggtctgtag 20

<210> SEQ ID NO 38

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 38

tatcaagctg tgcagagaca 20

<210> SEQ ID NO 39

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 39

caggaactcc cagggtggc 20

<210> SEQ ID NO 40

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 40

gctctgtatg tgtgtgtgcg 20

<210> SEQ ID NO 41

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 41

agatcccgga ttcggtcccc 20

<210> SEQ ID NO 42

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 42

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cggtgcgccca ttccctgccca 20

<210> SEQ ID NO 43  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 43

gggatagaga tttttgagct 20

<210> SEQ ID NO 44  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 44

gatctgggac ttggaggttg 20

<210> SEQ ID NO 45  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 45

tccaaggtca taagaaggca 20

<210> SEQ ID NO 46  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 46

atgatcagcc ggtcagacca 20

<210> SEQ ID NO 47  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 47

cccaggaatg ctgttctcca 20

<210> SEQ ID NO 48  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 48

tttcaggact tcattccagcc 20

<210> SEQ ID NO 49

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<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 49  
  
ccagcaggat ctccttggtg 20  
  
<210> SEQ ID NO 50  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 50  
  
tccagtgcctt tctgctccag 20  
  
<210> SEQ ID NO 51  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 51  
  
acagtgtctg aaagtagggc 20  
  
<210> SEQ ID NO 52  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 52  
  
gctggccctg ctagcacctc 20  
  
<210> SEQ ID NO 53  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 53  
  
ccacagagac atgatctggg 20  
  
<210> SEQ ID NO 54  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 54  
  
gtcttaaaact ttagttcttc 20  
  
<210> SEQ ID NO 55  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 55

tctagctctc cagtgtctc 20

<210> SEQ ID NO 56

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 56

ggccctgacc agcggaggct 20

<210> SEQ ID NO 57

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 57

cctctgtgac agactcagt 20

<210> SEQ ID NO 58

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 58

tccatactga ggctgttgtc 20

<210> SEQ ID NO 59

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 59

cctggcccg gatgacatg 20

<210> SEQ ID NO 60

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 60

gaaggcacca tggtaggcat 20

<210> SEQ ID NO 61

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 61

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ccaatccaag tgcctgagg 20

<210> SEQ ID NO 62  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 62

cagctgggat caccaactgg 20

<210> SEQ ID NO 63  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 63

gtgtctcaga gcctgaactt 20

<210> SEQ ID NO 64  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 64

taagcagtgg ctgccccagc 20

<210> SEQ ID NO 65  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 65

cctccctctt cagtgtaacg 20

<210> SEQ ID NO 66  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 66

agaagccttc catgccctaa 20

<210> SEQ ID NO 67  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 67

tatgttcctg cctatccgtc 20

<210> SEQ ID NO 68

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<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 68  
  
caactaaggt gccagctata 20  
  
<210> SEQ ID NO 69  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 69  
  
tggtcatgca actaaggtgc 20  
  
<210> SEQ ID NO 70  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 70  
  
atttgtgttg tcacgtaggc 20  
  
<210> SEQ ID NO 71  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 71  
  
tctcaccctc ccaaatttgt 20  
  
<210> SEQ ID NO 72  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 72  
  
agcacacttg ctgctgtcctt 20  
  
<210> SEQ ID NO 73  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide  
  
<400> SEQUENCE: 73  
  
gccaggcctg gaccagact 20  
  
<210> SEQ ID NO 74  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 74

gggcaacaga aaagatgcag 20

<210> SEQ ID NO 75

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 75

aatgtcagct tttaatctgt 20

<210> SEQ ID NO 76

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 76

gagtcaatgc ctgagatggg 20

<210> SEQ ID NO 77

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 77

caggaagcaa ctgggagtga 20

<210> SEQ ID NO 78

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 78

ccatctcaga gaaggcattg 20

<210> SEQ ID NO 79

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 79

tgcacatgtc cctgtgggat 20

<210> SEQ ID NO 80

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 80

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gggactcacc ggtcagacca 20

<210> SEQ ID NO 81  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 81

agtgttggt ccctggagga 20

<210> SEQ ID NO 82  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 82

agtccttac accatatctg 20

<210> SEQ ID NO 83  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 83

caaagtgtgg aagtgaagg 20

<210> SEQ ID NO 84  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 84

ctctggtggc cacggtggga 20

<210> SEQ ID NO 85  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 85

ggtgtatggc tgctcagact 20

<210> SEQ ID NO 86  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 86

aggaggtaca tgtgactgac 20

<210> SEQ ID NO 87

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<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 87

gacctgtcca ttcgctcact 20

<210> SEQ ID NO 88  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 88

tgagccagcc agtgacagcg 20

<210> SEQ ID NO 89  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 89

agcactggag aaatcatcaa 20

<210> SEQ ID NO 90  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 90

ttgggcttga ggttcctggg 20

<210> SEQ ID NO 91  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 91

gatccagatt tggaaacggc 20

<210> SEQ ID NO 92  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 92

agagctagag gcagccaaag 20

<210> SEQ ID NO 93  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 93

tgtcctggac ctcaccaaac 20

<210> SEQ ID NO 94  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 94

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aggagcctca cctgcagatg 20

<210> SEQ ID NO 95  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 95

ccacatggac ctaagggccca 20

<210> SEQ ID NO 96  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 96

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<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 97

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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 98

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<210> SEQ ID NO 99  
<211> LENGTH: 20  
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 99

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<210> SEQ ID NO 100  
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 100

tgagagcata tatcagaggg 20

<210> SEQ ID NO 101  
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<400> SEQUENCE: 101

acagtacgtt actagccttc 20

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<212> TYPE: DNA  
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<400> SEQUENCE: 103

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<211> LENGTH: 20  
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 104

aagatgtgtg aaactctgaa 20

<210> SEQ ID NO 105  
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<400> SEQUENCE: 105

ctacagacct atggggcctg 20

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<400> SEQUENCE: 106

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gccagccctg ggagttcctg 20

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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 108

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<210> SEQ ID NO 109  
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 111

caacctccaa gtcccagatc 20

<210> SEQ ID NO 112  
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<400> SEQUENCE: 113

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<400> SEQUENCE: 114

tgagagaacag cattcctggg 20

<210> SEQ ID NO 115  
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<400> SEQUENCE: 115

caacaaggag atcctgctgg 20

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ctggagcaga aagcactgga 20

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&lt;211&gt; LENGTH: 20

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&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 118

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&lt;210&gt; SEQ ID NO 119

&lt;211&gt; LENGTH: 20

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 119

gagaccactg gagagctaga 20

&lt;210&gt; SEQ ID NO 120

&lt;211&gt; LENGTH: 20

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 120

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&lt;210&gt; SEQ ID NO 121

&lt;211&gt; LENGTH: 20

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 121

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&lt;210&gt; SEQ ID NO 122

&lt;211&gt; LENGTH: 20

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 122

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&lt;210&gt; SEQ ID NO 123

&lt;211&gt; LENGTH: 20

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 123

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&lt;210&gt; SEQ ID NO 124

&lt;211&gt; LENGTH: 20

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&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 124

gcttacctg aagagggagg 20

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<210> SEQ ID NO 125  
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<400> SEQUENCE: 125

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<210> SEQ ID NO 126  
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<400> SEQUENCE: 126

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<210> SEQ ID NO 128  
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<400> SEQUENCE: 128

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<210> SEQ ID NO 129  
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<400> SEQUENCE: 129

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<400> SEQUENCE: 130

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<400> SEQUENCE: 131

agtctgggtc caggcctggc 20

<210> SEQ ID NO 132  
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 132

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<400> SEQUENCE: 133

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<210> SEQ ID NO 134  
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 134

atccccacagg gacatgtgca 20

<210> SEQ ID NO 135  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 135

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<210> SEQ ID NO 136  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 136

agtctgagca gccatacacc 20

<210> SEQ ID NO 137  
<211> LENGTH: 3790  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens  
<220> FEATURE:

<400> SEQUENCE: 137

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gagcaggagg ggtaaggatt ctgccttgag gagaaaagag ctggggcaac ctccaagtcc 120

cagatcatgt ctctgtgggg tctggtctcc aagatgcccc cagaaaaagt gcagcggctc 180

tatgtcgact ttccccaaca cctgcggcat cttctgggtg actggctgga gagccagccc 240

tgggagttcc tggtcggctc cgacgccttc tgcgtcaact tggctagtgc cctactttca 300

gacactgtcc agcaccttca ggcctcgggt ggagagcagg gggaggggag caccatcttg 360

caacacatca gcacccttga gagcatatat cagagggacc ccctgaagct ggtggccact 420

ttcagacaaa tacttcaagg agagaaaaaa gctgttatgg aacagtccg cacttgcca 480

atgcctttcc actggaagca ggaagaactc aagtttaaga caggcttgcg gaggtgcag 540

caccgagtag gggagatcca cttctccga gaagccctgc agaagggggc tgaggtggc 600

caagtgtctc tgcacagctt gatagaaact cctgctaatg ggactgggcc aagtgaggcc 660

ctggccatgc tactgcagga gaccactgga gagctagagg cagccaaagc ctagtgctg 720

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| gagagcctgg | ccccactcca | ggagagggtg | gaaagcctgg  | tggacattta | ttcccagcta | 840  |
| cagcaggagg | tagggggcgc | tgggtgggag | cttgagccca  | agacccgggc | atcgctgact | 900  |
| ggccggctgg | atgaagtcc  | gagaaccctc | gtcaccagtt  | gcttctgggt | ggagaagcag | 960  |
| ccccccagg  | tactgaagac | tcagaccaag | ttccaggctg  | gagttcgatt | cctgttgggc | 1020 |
| ttgaggttcc | tgggggcccc | agccaagcct | ccgctggcca  | gggccgacat | ggtgacagag | 1080 |
| aagcaggcgc | gggagctgag | tgtgcctcag | ggcctggggg  | ctggagcaga | aagcactgga | 1140 |
| gaaatcatca | acaacactgt | gcccttggag | aacagcattc  | ctgggaactg | ctgctctgcc | 1200 |
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| gagaacatcc | agccattctc | tgccaaagac | ctgtccattc  | gctcactggg | ggaccgaatc | 1860 |
| cgggatcttg | ctcagctcaa | aaatctctat | cccaagaagc  | ccaaggatga | ggctttccgg | 1920 |
| agccactaca | agcctgaaca | gatgggtaag | gatggcaggg  | gttatgtccc | agctaccatc | 1980 |
| aagatgacgc | tggaaaggga | ccaaccactt | cctaccccag  | agctccagat | gcctaccatg | 2040 |
| gtgccttctt | atgaccttgg | aatggcccct | gattcctcca  | tgagcatgca | gcttggccca | 2100 |
| gatatggtgc | cccagggtga | cccaccacac | tctcactcca  | tccccccgta | tcaaggcctc | 2160 |
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| cccagcctgg | gccagatgag | cctgcctctt | gaccagcctc  | acccccaggg | cctgctgccc | 2280 |
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| atggtggaag | acagctgcct | gagccagcca | gtgacagcgt  | ttcctcaggg | cacttggatt | 2400 |
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| tcccactatg | ggcaatctgg | gatctcaatg | tcccacatgg  | acctaagggc | caaccccagt | 2580 |
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| ccacctactc | ctgggtcagg | aggaaaagac | taacaggaga  | atgcacagtg | ggtggagcca | 2760 |
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| aagttcaggc | tctgagacac | gccccaacat | gcctgcacct  | gcagcgcgca | cacgcacgca | 2880 |
| cacacacata | cagagctctc | tgagggtgat | ggggtgagc   | aggagggggg | ctgggtaaga | 2940 |
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| tgtagccag gtggaggctt ctgatactgt tatgagacta tgcaaaagta caagggctga   | 3240 |
| gattcttcgt gtatagctgt gtgaacgtgt atgtacctag gatatgttaa atatatagt   | 3300 |
| ggcaccttag ttgcatgacc acatagaaca tgtgtctatc tgcttttgcc tacgtgacaa  | 3360 |
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| agtggctgtg acatctactgt atggctccac acctccaatg ctgcctggga gccagggtga | 3540 |
| gagtctgggt ccaggctctg ccattgtggc ctccagtgtg tgagagggcc ctgcctgctg  | 3600 |
| catcttttct gttgccccat ccaccgccag cttcccttca ctcccctatc ccattctccc  | 3660 |
| tctcaaggca ggggtcatag atcctaagcc ataaaataaa ttttattcca aaataacaaa  | 3720 |
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&lt;210&gt; SEQ ID NO 138

&lt;211&gt; LENGTH: 3667

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&lt;213&gt; ORGANISM: Homo sapiens

&lt;220&gt; FEATURE:

&lt;400&gt; SEQUENCE: 138

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| gatgccccca gaaaaagtgc agcggctcta tgtcgacttt ccccaacacc tgcggcatct | 180  |
| tctgggtgac tggctggaga gccagccctg agcatatata agagggaccc cctgaagctg | 240  |
| gtggccactt tcagacaaat acttcaagga gaaaaaaaag ctgttatgga acagttccgc | 300  |
| cacttgccaa tgcttttcca ctggaagcag gaagaactca agtttaagac aggcttgctg | 360  |
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| acatgtgtct atctgctttt gcctacgtga caacacaaat ttgggagggt gagacactgc  | 3300 |
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| aatttgaggt ctgactgtgc ccaagtgggt ccaagtggct gtgacatcta cgtatggctc  | 3420 |
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|                                                                   |      |
|-------------------------------------------------------------------|------|
| gccctccagt gtagagagg gccctgcctg ctgcattctt tctgttgccc catccaccgc  | 3540 |
| cagcttccct tcactccct atccattct cctctcaag gcaggggtca tagatcctaa    | 3600 |
| gccataaaat aaattttatt ccaaaataaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa | 3660 |
| aaaaaaa                                                           | 3667 |

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What is claimed is:

1. A compound 8 to 80 nucleobases in length targeted to a nucleic acid molecule encoding STAT 6, wherein said compound specifically hybridizes with said nucleic acid molecule encoding STAT 6 (SEQ ID NO: 4) and inhibits the expression of STAT 6.

2. The compound of claim 1 comprising 12 to 50 nucleobases in length.

3. The compound of claim 2 comprising 15 to 30 nucleobases in length.

4. The compound of claim 1 comprising an oligonucleotide.

5. The compound of claim 4 comprising an antisense oligonucleotide.

6. The compound of claim 4 comprising a DNA oligonucleotide.

7. The compound of claim 4 comprising an RNA oligonucleotide.

8. The compound of claim 4 comprising a chimeric oligonucleotide.

9. The compound of claim 4 wherein at least a portion of said compound hybridizes with RNA to form an oligonucleotide-RNA duplex.

10. The compound of claim 1 having at least 70% complementarity with a nucleic acid molecule encoding STAT 6 (SEQ ID NO: 4) said compound specifically hybridizing to and inhibiting the expression of STAT 6.

11. The compound of claim 1 having at least 80% complementarity with a nucleic acid molecule encoding STAT 6 (SEQ ID NO: 4) said compound specifically hybridizing to and inhibiting the expression of STAT 6.

12. The compound of claim 1 having at least 90% complementarity with a nucleic acid molecule encoding STAT 6 (SEQ ID NO: 4) said compound specifically hybridizing to and inhibiting the expression of STAT 6.

13. The compound of claim 1 having at least 95% complementarity with a nucleic acid molecule encoding STAT 6 (SEQ ID NO: 4) said compound specifically hybridizing to and inhibiting the expression of STAT 6.

14. The compound of claim 1 having at least one modified internucleoside linkage, sugar moiety, or nucleobase.

15. The compound of claim 1 having at least one 2'-O-methoxyethyl sugar moiety.

16. The compound of claim 1 having at least one phosphorothioate internucleoside linkage.

17. The compound of claim 1 having at least one 5-methylcytosine.

18. A method of inhibiting the expression of STAT 6 in cells or tissues comprising contacting said cells or tissues with the compound of claim 1 so that expression of STAT 6 is inhibited.

19. A method of screening for a modulator of STAT 6, the method comprising the steps of:

a. contacting a preferred target segment of a nucleic acid molecule encoding STAT 6 with one or more candidate modulators of STAT 6, and

b. identifying one or more modulators of STAT 6 expression which modulate the expression of STAT 6.

20. The method of claim 19 wherein the modulator of STAT 6 expression comprises an oligonucleotide, an antisense oligonucleotide, a DNA oligonucleotide, an RNA oligonucleotide, an RNA oligonucleotide having at least a portion of said RNA oligonucleotide capable of hybridizing with RNA to form an oligonucleotide-RNA duplex, or a chimeric oligonucleotide.

21. A diagnostic method for identifying a disease state comprising identifying the presence of STAT 6 in a sample using at least one of the primers comprising SEQ ID NOS: 5 or 6, or the probe comprising SEQ ID NO: 7.

22. A kit or assay device comprising the compound of claim 1.

23. A method of treating an animal having a disease or condition associated with STAT 6 comprising administering to said animal a therapeutically or prophylactically effective amount of the compound of claim 1 so that expression of STAT 6 is inhibited.

24. The method of claim 23 wherein the disease or condition is an autoimmune disorder.

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